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Large-Scale Conversion of Livestock Blood into Amino Acid Liquid Fertilizer and Dry Protein Feedstuff: A Case Study

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Abstract: Livestock blood, typically considered a waste byproduct of the slaughter industry, has the potential to be a valuable resource in the environmental and agricultural industries owing to its high protein content. This study reports the mechanisms involved in developing a continuous process capable of processing 5 tons/day of livestock blood into high purity amino acid liquid fertilizer and dried protein feedstuff simultaneously. Large-scale processing units were fabricated for the ultrasonic pretreatment and solubilization of proteins, enzymatic degradation of dissolved proteins for amino acid conversion, solid-liquid separation using a membrane filter press to produce high purity amino acid liquid fertilizer, and microwave drying of the solid component to produce dry protein feedstuff. The main processing units were integrated into a continuous, efficient system. The final amino acid liquid fertilizer and dry protein feedstuff contained >20% amino acids and approximately 78% protein, respectively. An economic feasibility analysis of the integrated system based on an annual processing capacity of 3000 tons of livestock blood yielded a total annual profit of 17.4 million euros (5812 euros/ton). This study presents an efficient and profitable approach to repurposing the waste generated by slaughterhouses toward agriculture and feed production.

Keywords: amino acid liquid fertilizer; dried protein feed; enzymatic degradation; filter press; microwave drying; ultrasonic solubilization; slaughter blood

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1. Introduction

Livestock blood is among the various organic wastes generated during the slaughtering of livestock. Driven by the increasing consumption of meat due to improving living standards and incomes, there is a rising trend in the number of livestock slaughtered and the volume of livestock blood generated. In South Korea, the majority of livestock blood, except for a small amount used in the food industry, was originally disposed of through marine discharge. However, since 2013, marine discharge has been prohibited by the revised Prevention of Marine Pollution Act following the commencement of the "1996 Protocol", which greatly strengthened marine discharge regulations. Consequently, most of the livestock blood generated in South Korea is now treated as wastewater [1]. Notably, a small portion of livestock blood is commonly used in the production of medical bioadhesives [2] and immunoglobulins [3]. In addition, it has also been utilized as fertilizer or feed [4]. However, the large-scale commercial production of fertilizer from livestock blood remains challenging. A major disadvantage of using livestock blood as a raw material is the mandatory requirement for wastewater treatment, necessitating the construction of additional processing units [3,5].

Livestock blood is dark brown in color and consists of water (~80%), and organic matter (19–20%), which is comprised mostly of proteins (accounting for more than 90% of organic matter), making it a valuable waste resource [6]. Blood plasma contains proteins such as albumin, globulin, and fibrinogen, while blood cells contain hemoglobin. Since blood cells have a higher protein content than blood plasma, most proteins can only be

accessed by processing blood cells through mechanisms like ultrasonic solubilization [7]. Subsequently, the ultrasonically solubilized livestock blood can be further degraded into amino acids via protease treatments.

The consumption of eco-friendly agricultural products has rapidly increased in recent decades due to improved health awareness, food safety standards, and environmental awareness. This increase has stimulated the growth of eco-friendly agriculture, which relies heavily on eco-friendly pesticides and fertilizers [8]. Eco-friendly fertilizers refer to organic fertilizers produced using organic matter, such as livestock manure, food waste, and livestock blood [9]. Among such eco-friendly fertilizers are organic amino acid fertilizers, which have numerous beneficial effects on crop production [10], such as promoting plant growth, enabling normal functioning under cold or dark conditions, enhancing pathogen resistance, boosting fruit sugar and trace element contents, improving stress resistance, and maintaining soil health. Liu et al. [11] realized the industrial production of amino acid liquid fertilizer using yak blood and evaluated its effect on promoting growth, increasing yield, and improving the quality of agricultural products by planting spinach in a greenhouse. In their study, amino acid liquid fertilizer was manufactured through enzymatic hydrolysis of yak blood, and it was reported that there was no significant difference ($p > 0.05$) in efficacy compared to commercially purchased amino acid liquid fertilizer. Particularly, it showed the most superior effects in terms of stem diameter enhancement, nitrate content, and vitamin C content compared to other fertilizer groups.

This study aimed to develop a continuous process to simultaneously produce amino acid liquid fertilizer (for crop production) and dried protein feedstuff (for livestock production) from livestock blood using large-scale processing units. However, livestock blood is difficult to handle due to its high environmental loads and its rapid decay rate. We strived to extract all proteins through ultrasonic solubilization and subsequently convert them into amino acids through enzymatic degradation. Following which, solid-liquid separation was performed to extract both the liquid and solid components, which were then utilized to produce amino acid liquid fertilizer and protein feedstuff, respectively. The large-scale processing units included a protein solubilization ultrasound device, enzymatic degradation reactor, membrane filter press for solid-liquid separation, and a microwave dryer.

We targeted a treatment capacity of 5 tons/day for our continuous process, with the applicability at a commercial scale as viable starting from a minimum level of 5 tons/day or more. Therefore, in this paper, we focused on presenting the concept of process design, the scale-up process, and the operation and economic evaluation of the full-scale system after scale-up, rather than detailing the specific lab experiment results of each individual process. Through this approach, we aimed to confirm the feasibility of sustainable conversion of livestock blood into agricultural resources at a minimum commercial scale.

2. Materials and Methods

2.1. Preparation of Raw Livestock Blood Samples

This study utilized pig blood obtained from a local slaughterhouse. The typical pig slaughter process in South Korea involves about 26 steps, with most of the livestock blood generated during the bleeding process. Blood volume accounts for approximately 3.5% of total body weight, and bleeding time is <5 min. The blood samples were delivered to the laboratory under refrigeration within 30 min, then homogenized, divided into smaller portions, and stored in a refrigerator (4 °C).

2.2. Concepts and Method for Process Design

2.2.1. Solubilization of Livestock Blood

First, for the development of the process, the primary goal was to convert the proteins in livestock blood into amino acids using protease enzyme. However, the enzymatic degradation of livestock blood is very inefficient due to the resistance presented by the cell wall [12,13]. Therefore, in this study, we attempted to extract most of the proteins

contained in the livestock blood through ultrasonic solubilization and effectively convert the dissolved proteins to amino acids by enzymatic degradation.

Since blood starts to coagulate the moment it leaves the body, we added a simple grinding process to homogenize the coagulated blood before ultrasonic solubilization. This process refers to cavitation, which involves the formation and implosion of microbubbles in a fluid occurring under large localized negative pressures. Cavitation generates high temperatures and pressures that cause pyrolysis and oxidation through the production of -OH radicals. Additionally, cavitation can enhance the rate of various chemical reactions by promoting collisions between molecules [14]. Ultrasound technology is also widely used to treat water, soil, air, and waste. Figure 1 shows a bench-scale ultrasonic pretreatment unit (Anytech, Suwon, South Korea) that was used to plan the scale-up design. The entire system is largely composed of an ultrasonic generator and an ultrasonic reactor, along with a metering pump, as well as input and output vessels for continuous operation. We selected a probe-type ultrasonic reactor because of its high versatility and easy operation. The ultrasonic reactor was composed of an ultrasonic transducer, reaction vessel, thermocouple, constant-temperature water bath, and agitator for stirring. The reaction vessel was a 2 L beaker, but the experimental capacity was set to 1 L/batch considering the effective volume. An agitator could operate at a maximum speed of 1000 rpm. The ultrasonic transducer consisted of a converter, horn, and cooling fan. Three ultrasonic oscillators of 20, 24, and 28 kHz were used for effective cell wall destruction. Experiments were conducted to optimize the ultrasonic frequency, density, time, and agitation speed based on the protein solubilization rate.

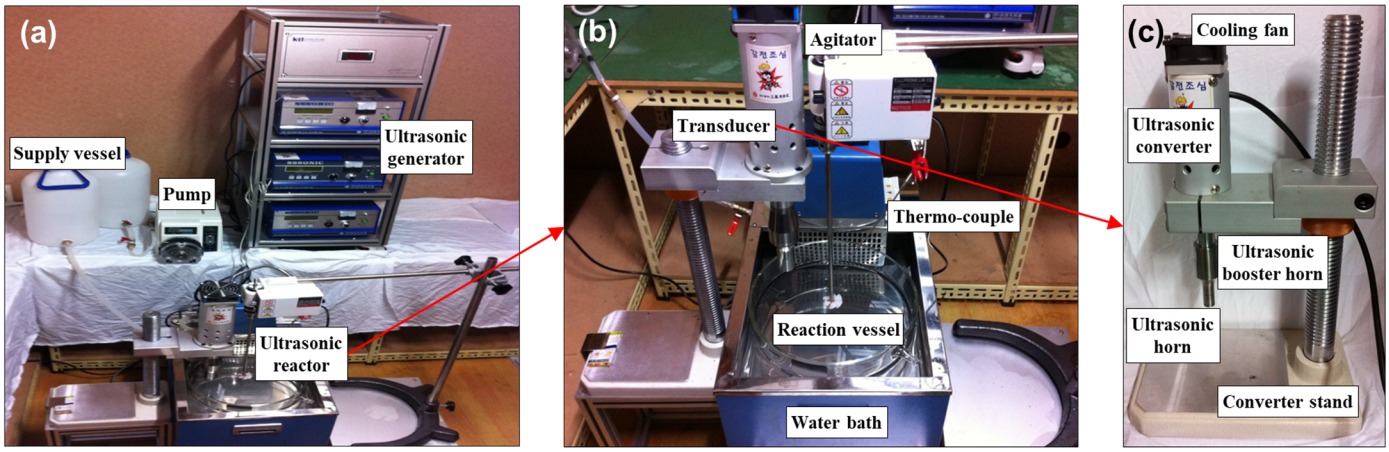


Figure 1. Bench-scale ultrasonic pretreatment system used to derive the design for the large-scale unit: (**a**) Entire system; (**b**) Ultrasonic reactor; (**c**) Transducer.

2.2.2. Conversion of Proteins in Livestock Blood into Amino Acids

The ultrasonically pretreated livestock blood was transferred to the enzymatic reactor, which maintains the agitation speed and reaction temperature to ensure good mixing and effective protein breakdown. The efficiency of enzymatic degradation is highly dependent on the type and amount of enzyme, substrate, reaction temperature, and agitation method used [13]. Therefore, we conducted a preliminary bench-scale experiment to select the optimal reaction time, temperature, agitation method, and enzyme type and amount based on the degree of protein degradation and amino acid production. We mainly used two endo-type enzymes and one exo-type enzyme procured from Novozymes (Frederiksberg, Denmark) and an 8-L reactor. The capacity of bench-scale experiment was based on 3 L/batch. The reactor for enzymatic degradation was a double-jacketed vessel that uses a water bath to enable hot water supply and circulation and maintain a constant temperature within the reactor during enzymatic reactions. To ensure that the samples are evenly mixed during enzymatic reactions, four baffles were installed on the inner walls of the reactor.

2.2.3. Solid-Liquid Separation for the Production of High-Quality Amino Acid Liquid Fertilizer

To produce high purity amino acid liquid fertilizer from the enzymatic degradation reactants, we separated the solid and liquid components of the blood using a filter press. In a typical filter press, the slurry-like substance is pressurized and dewatered using a pump [15]. The filter plates of the filter press are supported by a hydraulic system, and the slurry injected through the inlet is pressurized by the pump. The filter cloth on the surface of the filter plate acts as a filter, and the filtrate is discharged to the outside through a groove. We incorporated a secondary process of filtration using the membrane technique—referring to the secondary pressurized dewatering of the slurry by swelling of the membrane filter plate surface. The membrane technology has a shorter filtration time and higher filtration efficiency than the traditional pressurized filter press technology [15]. The optimal solid-liquid separation conditions, including filter cloth type, primary feeding pressure, and secondary dewatering pressure, for producing high-quality amino acid liquid fertilizer were determined via a bench-scale experiment using the membrane filter press (Dongil Canvas Engineering, Pyeongtaek, South Korea) (Figure 2). We established the optimal coagulation conditions separately. The filtration area and volume were 0.21 m^2 and 1.2 L, respectively, and five filter plates were used. The sample injection capacity for the experiment was 1.02 L/min. The press applied a maximum pressure of 2 MPa.

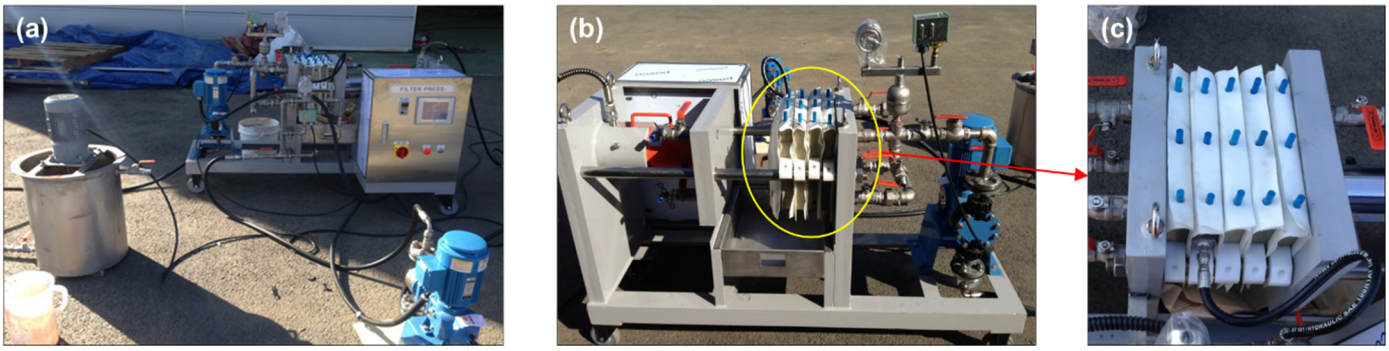


Figure 2. Bench-scale membrane filter press system used to derive the design for the large-scale unit: (**a**) Entire system (front view); (**b**) Main body (back view); (**c**) Filter plate.

2.2.4. Drying for the Production of Dry Protein Feedstuff

The solid-liquid separation process produces not only a high purity amino acid liquid fertilizer but also a semi-solid dewatered blood cake. We predicted that this blood cake would contain high amounts of protein, and, therefore, converted this by-product into protein feedstuff. The residual moisture stimulates the rapid deterioration of the dewatered cake during transportation. Therefore, we applied microwave drying to remove the residual moisture. Microwave heating has several advantages over traditional heating methods, such as hot air [16], non-contact heating, efficient energy transfer, rapid heating, selective material heating, volumetric heating, quick start-up and termination, targeted heating of sample interior, and a higher level of safety and automation.

We designed the microwave drying procedure based on the procedure used to sterilize livestock feed. Microwave irradiation can destroy cells by generating friction and heat, electron ion motion, and electric field effects caused by the rotational vibration of molecules, which provides an instantaneous sterilization effect. Experiments were performed to optimize the drying time, microwave irradiation height, and thickness of the sample. We used a 4-kW batch-type bench-scale microwave unit (Serok, Busan, South Korea) (Figure 3), which was designed to vary the power and irradiation height of the microwave. A bench-scale microwave dryer largely consists of a drying chamber and a microwave irradiation section. Four magnetrons with a capacity of 1 kW that irradiate microwaves at a frequency of 2.45 GHz are applied, allowing the microwave output to be up to 4 kW. The experiment was conducted under the condition of 4 kW magnetron output, which is the maximum

output of the dryer, and the input amount of dewatered cake was 4 kg/batch. Based on the optimization experiments, a large-scale microwave dryer was designed and fabricated.

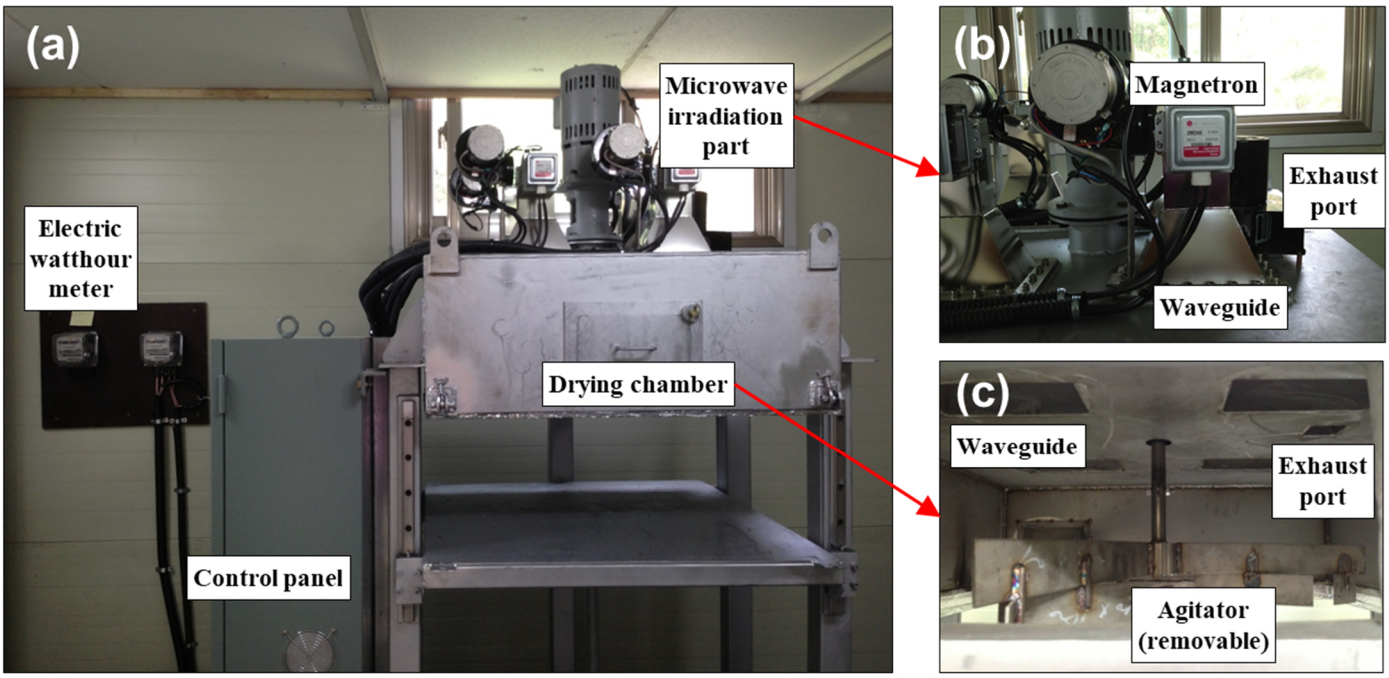


Figure 3. Bench-scale microwave dryer system used to derive the design for the large-scale unit: (**a**) Entire system; (**b**) Microwave irradiation section (top of dryer); (**c**) Inside the drying chamber.

2.3. Physicochemical Analysis

We analyzed the moisture, organic, and inorganic contents of the pig blood using the Standard Methods, with the analysis temperature set at 105 °C for moisture and 550 °C for organic matter [17]. In addition, we measured the salt content using Standard Methods and the total lipid content using the sulfo-phospho-vanillin reaction method [18]. The blood proteins were analyzed to determine the total protein, albumin, fibrinogen, platelet, and hemoglobin counts using an automatic hematology analyzer (Coulter Ac•T; Beckman Coulter, Carlsbad, CA, USA) [19]. Total C, H, O, N, and S were determined in sieved samples after drying with a FLASH-2000 auto-analyzer (Thermo Fisher Scientific, Bremen, Germany). The concentrations of heavy metals (As, Cd, Hg, Pb, Cr, Cu, Ni, and Zn) were determined using an inductively-coupled plasma optical emission spectrometer (ICP-OES) (Optima 2100DV, Perkin Elmer, Waltham, MA, USA). Additionally, to determine whether any potential pathogens present in the pulmonary blood were eliminated, the dry rehydratable film method (3M™ Petrifilm, NEOGEN, Lansing, MI, USA) was applied to analyze the general bacteria and pathogenic *Escherichia coli*, while *Salmonella* sp. and *Bacillus* sp. were analyzed using the standard plate culture method [20].

The proteins and amino acids were analyzed using high performance liquid chromatography (HPLC), gel permeation chromatography (GPC), and matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF). We analyzed the amino acid conversion efficiency based on the changes in free amino acids and peptides. Analysis of the free amino acids was performed using HPLC (Zorbax Eclipse-AAA Columns, Agilent, Santa Clara, CA, USA) after precipitating the proteins by adding 10% (v/v) tricarboxylic acid to the centrifuged supernatant. The peptides were analyzed using GPC (1100 HPLC, Agilent), which measures the differences in the filtering speeds of samples with various sizes. The experiments were conducted using a Phenomenex BioSep-SEC-S 2000 column (300 × 7.8 mm, 5 microns) with 20 mM phosphate buffer as the mobile phase. The flow rate was 0.45 mL/min, the injection volume was 10–50 µL, and the total measurement time was 45 min. The MALDI-TOF analysis (Ultraflex III, Bruker, Mannheim, Germany) was also

performed to measure the absolute mass of the proteins and peptides. For this procedure, the laser repetition rate was set at 20 Hz, the extraction delay time at 575 ns, and the number of laser shots at 500/spectrum. The solubilization rate (%) using the ultrasonic pretreatment was calculated using the obtained hemoglobin concentration and the following equation:

$$SR\ (\%) = (Ht - Hu)/(Hw - Hu) \times 100,$$

where *SR* is the solubilization rate, *Ht* is the plasma hemoglobin in treated blood, *Hu* is the plasma hemoglobin content in untreated blood, and *Hw* is the whole-blood hemoglobin content.

3. Results and Discussion

3.1. Characteristics of Livestock Blood Used in This Study

Each analysis was performed five times, and the data are presented as the mean and standard deviation (Table 1). We analyzed the moisture, organic matter (volatile solid, VS), and inorganic matter (fixed solid, FS) content of the pig blood. The content of average moisture, organic matter, and inorganic matter was 78.99%, 19.72%, and 1.29%, respectively, consistent with previous findings [12]. The total protein content was 18.30%, indicating that most of the organic matter content was composed of protein. The proteins in the livestock blood are primarily comprised of hemoglobin (14.05% of blood content), along with albumin, globulin, and fibrinogen.

Table 1. Characteristics of the livestock blood used in this study.

Parameter	Value [1]
Moisture (wt%)	78.99 (0.15)
Organic matter (wt%)	19.72 (0.23)
Protein (wt%)	18.30 (0.09)
Albumin (wt%)	2.04 (0.11)
Globulin (wt%)	2.10 (0.10)
Fibrinogen (wt%)	0.11 (0.03)
Hemoglobin (wt%)	14.05 (0.16)
Inorganic matter (wt%)	1.29 (0.30)
Lipid (wt%)	0.17 (0.01)
Salt (wt%)	0.96 (0.05)
pH	7.14 (0.08)
Carbon (wt%, d.b. [2])	45.00 (1.67)
Hydrogen (wt%, d.b.)	6.21 (0.21)
Oxygen (wt%, d.b.)	17.60 (1.03)
Nitrogen (wt%, d.b.)	12.65 (0.70)
Sulfur (wt%, d.b.)	0.14 (0.03)
Arsenic (mg/kg)	<LOD [3]
Cadmium (mg/kg)	<LOD
Mercury (mg/kg)	<LOD
Lead (mg/kg)	0.02 (0.02)
Chrome (mg/kg)	0.05 (0.03)
Copper (mg/kg)	2.67 (1.35)
Nickel (mg/kg)	<LOD
Zinc (mg/kg)	6.75 (0.45)
Total Colony Counts (CFU [4]/mL)	3.8×10^7
Total Coliforms (CFU/mL)	2.7×10^5
Salmonella (CFU/mL)	N.D. [5]
Bacillus (CFU/mL)	4.1×10^8

[1] Mean with standard deviation in parentheses. [2] d.b., dry basis. [3] LOD, limit of detection. [4] CFU, colony forming unit. [5] N.D., not detected.

The average lipid content was 0.17%, which was similar to the previously reported approximately 0.2% [13]. The average salt content was approximately 0.96%. Salt content is a particularly important property of fertilizers because it can accumulate in soil and interfere with the adsorption of nutrients to the soil particles, causing a loss of nutrients and interfering with the absorption of nutrients and moisture by plants. High salt concentrations cause osmotic disorders that destroy cell tissues and promote the incidence of disease [21]. Notably, the average salt content in pig blood was below the limit (1.0%) prescribed by the Korean fertilizer quality standard and thus did not require a separate process for salt removal.

Elemental analysis showed that carbon had the highest average content (45.00%), followed by oxygen, nitrogen, hydrogen, and sulfur. Arsenic, cadmium, mercury, and nickel were not detected, while only trace amounts of lead, chromium, copper, and zinc were detected. The absence of these heavy metals reduces the risk of soil pollution, impairment of root development and crop growth, and risk of acute or chronic diseases [22]. Therefore, we concluded that livestock blood was safe to use as a raw material in the production of fertilizer.

In South Korea, livestock blood is commonly collected in an open system where carcasses are hung. The open system is associated with a high risk of contamination by intestinal contents and pathogens, such as *Salmonella* spp., *Escherichia coli*, *Shigella* spp., and *Yersinia enterocolitica* [23]. Pathogens can generate odors and inhibit amino acid conversion during enzymatic degradation, and some species exhibit high resistance, leading to various adverse effects such as gas generation. In countries where livestock blood is actively used, a closed-drainage system using a hollow knife is usually utilized. The hollow knife is inserted directly into the carotid artery to minimize microbiological contamination during collection [24].

We verified the presence of general bacteria, total coliforms, and *Bacillus* spp., and the absence of *Salmonella* spp. via bacterial culture experiments. Therefore, pre- and post-enzymatic degradation treatments are crucial to eliminate harmful microorganisms.

3.2. Unit Process Design

3.2.1. Ultrasonic Pretreatment for Protein Solubilization

We explored the optimal operating conditions (i.e., ultrasonic frequency, density, and time) for ultrasonic pretreatment of the livestock blood. First, the optimal ultrasonic frequency was determined from three frequencies: 20, 24, and 28 kHz. Then, the optimal ultrasonic density and time conditions were determined. Finally, ultrasonic density was limited to a maximum of 2 W/mL to account for operating costs.

The increase in temperature generated by cavitation promotes protein solubilization [10] and subsequent enzymatic degradation. However, protein denaturation above a certain temperature may lead to blood coagulation. Thus, we investigated the changes in the temperature of the target material according to the treatment time for each frequency. The highest temperature increase was observed at a frequency of 20 kHz. After 45 min at 20 kHz, the blood coagulated at a temperature of 69 °C. Accordingly, the internal temperature of the target material was maintained below 60 °C in subsequent ultrasonic pretreatment experiments to prevent blood coagulation.

Next, we investigated the solubilization rate of hemoglobin before and after pretreatment under each ultrasonic frequency. We found that hemoglobin concentration was highest at 20 kHz, and the protein solubilization rate decreased as the ultrasonic frequency increased. Consequently, 20 kHz was determined as the optimal ultrasonic frequency, which is consistent with previous findings in sewage sludge solubilization [14]. At 20 kHz, we varied ultrasonic density from 0.1 to 2.0 W/mL and ultrasonic time from 0 to 60 min to investigate the optimal pretreatment characteristics. The optimal ultrasonic density and time were determined as 0.5 W/mL and 10–30 min, respectively, depending on the treatment goal.

We also analyzed the changes in the livestock blood under the optimal ultrasonic pretreatment conditions. The hemoglobin concentration in the plasma of untreated and treated livestock blood was 0.03% and 15.35%, respectively, which corresponds to a solubilization rate of 97.72% under optimal pretreatment conditions. We also showed that the optimal pretreatment conditions killed 99.93% and 100% of general bacteria and total coliforms, respectively.

Based on the bench-scale ultrasonic pretreatment study, we fabricated a large-scale ultrasonic pretreatment unit with a treatment capacity of 5000 L/day. The unit features an actual ultrasonic reactor volume of 200 L/batch, an ultrasonic treatment time of 10 min/batch, a daily operating time of 600 min, 30 batches of treatments per day, and an ultrasonic density of 0.5 W/mL (Figure 4). The agitation speed was adjustable up to 90 rpm, and the ultrasonic oscillator (horn) consisted of five pieces to ensure uniform irradiation in the reactor.



Figure 4. Large-scale ultrasonic pretreatment unit following a scale-up design.

3.2.2. Enzymatic Degradation of Proteins to Amino Acids

We initially explored the flow characteristics in the reactor using computational fluid dynamics (CFD) analysis to determine the optimal reactor shape based on the agitator shape and presence or absence of a baffle. More specifically, we conducted an analysis of steady state and non-steady state for a total of three reactor types: disc-type impeller without baffles, blade-type impeller without baffles, and blade-type impeller with baffles. The analysis results showed that the reactor with the blade-type impeller incorporating baffles reached a uniform temperature in the shortest time. Next, we investigated the optimal temperature and agitation method for enzymatic degradation. After a 4-h enzymatic reaction involving 3 L of livestock blood at 50 °C, the protein concentration decreased from 239.85 to 42.50 g/L. Most of the reaction occurred within 2 h. However, the decrease in protein concentration does not necessarily correspond with amino acid conversion efficiency. Thus, we assessed the conversion efficiency through amino acid analysis. Amino acid concentrations increased from 2.07 to 107.89 g/L after 140 min. After that, amino acid concentrations decreased very slightly and then slightly increased after 200 min, indicating that the conversion of proteins to peptides and amino acids was completed within 140 min. CFD analysis showed that a steady reaction state was reached at approximately 90 min.

Consequently, the optimal reaction time for large-scale reactor operation was predicted to be approximately 2 h.

Based on the bench-scale experiments, we fabricated a large-scale enzyme reactor with a volume of 1.2 m^3 and a daily treatment capacity of 5 m^3 (Figure 5). The enzyme reaction time was 2 h/batch, the daily operating time was 600 min, and the daily processing frequency was 5 batches/day.

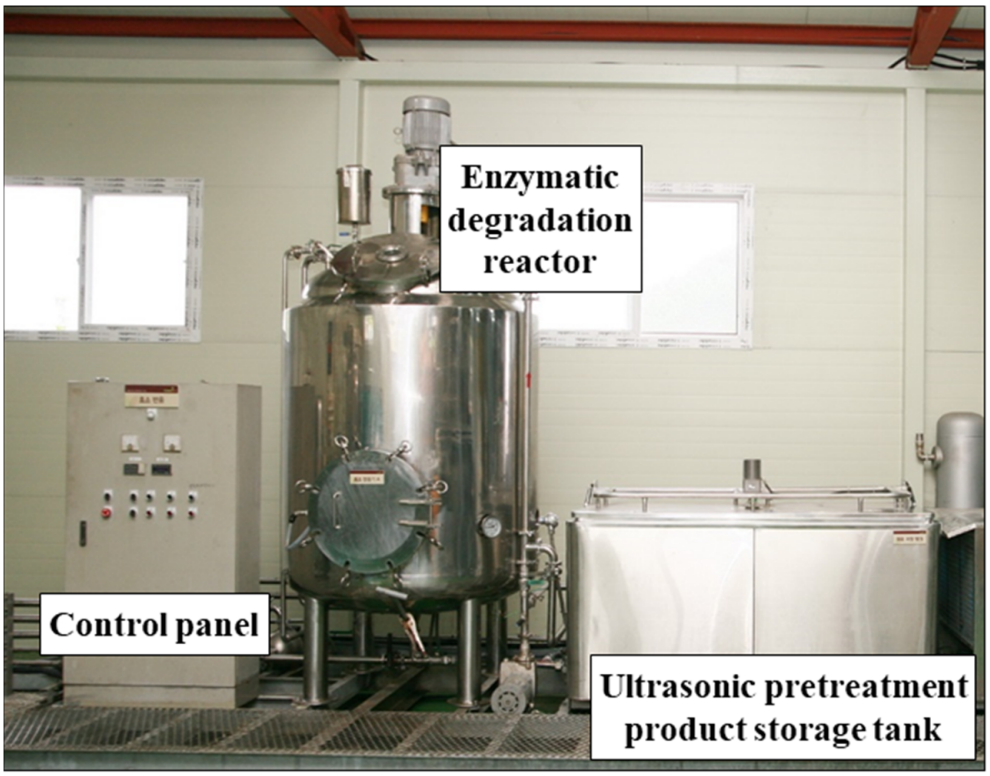


Figure 5. Large-scale enzymatic degradation system following a scale-up design.

We also investigated the optimal protease and degradation conditions. We selected two endo-type (Savinase®, Alcalse®) and one exo-type (Flavourzyme®) enzyme(s) from among 11 commercial proteases via a basic degradation characterization experiment. The albumin and hemoglobin proteolysis experiment revealed that the degree of proteolysis varied with enzyme type (exo-type > endo-type). A greater degree of proteolysis was achieved using a combination of enzymes because endo-type enzymes had little effect when used alone. Exo-type enzyme activity was stimulated by mixing with inexpensive endo-type enzymes. We explored the optimal enzyme combination for protein degradation and amino acid production in a preliminary bench-scale enzymatic degradation experiment. The optimal enzymatic degradation conditions were found with Savinase® 1% (*w*/*v*) at 60 °C over 1 h, followed by Flavourzyme® 0.1% (*w*/*v*) for >1 h. The total reaction time was set at 2–4 h, depending on the target amino acid concentration.

3.2.3. Solid-Liquid Separation Using Membrane Filter Press

We tried to selectively filter and isolate only high-quality free amino acids from the enzymatic degradation reaction products using membrane filter press technology. The properties of the enzymatic degradation reaction product were analyzed beforehand to confirm the applicability of the filter press. The pH of the enzymatic degradation reaction product was 7.08, with approximately 80% moisture and 19.5% organic matter content. Protein content was reduced to approximately 5.8 g/L via the enzymatic reaction, and amino acids were observed at 77.8 g/L, accounting for approximately 8%. The average particle diameter of the enzymatic degradation reaction product was 8.84 μm, with particles

ranging in size from 1 to 20 μm. This particle size distribution suggests that the filter cloth should ideally have a pore size of less than a few micrometers.

To improve the solid-liquid separation efficiency of the filter press, we added a chitosan coagulation step to the front-end process. The optimal injection rate of chitosan was found to be 0.05% (w/v) via a jar test. Chitosan is a natural high-molecular-weight polysaccharide that is tasteless, odorless, and harmless to the human body. Since chitosan is mainly extracted from red crab, it has no depletion potential, is easily biodegradable, and is environmentally friendly, non-toxic, and safe. In addition, because chitosan has an amino group, it is cationic in an aqueous solution, where it binds to anions and coagulates [15]. Chitosan is used as a coagulant for wastewater treatment and protein separation and recovery. In addition, chitosan has shown antibacterial and antifungal, preservative, deodorizing, and growth promotion effects in plants [25].

The filtration and dewatering experiment using the bench-scale membrane filter press was conducted on the enzymatic degradation reaction product coagulated using chitosan. The process selectively separated undegraded proteins and free amino acids from the enzymatic degradation reaction product, thereby producing high-quality amino acid liquid fertilizer and dewatered blood cake. Subsequently, we designed a large-scale membrane filter press and selected the optimal conditions based on the preliminary experiments conducted using the bench-scale membrane filter press. The large-scale system consisted of a total of 8 filtration chambers with 9 filter plates and a daily treatment capacity of a maximum of 5000 L with a filter volume of 192 L. The large-scale membrane filter press (Figure 6) consisted of a filter press, control panel, feed pump, chemical dissolution tank, chemical supply pump, pressing/washing water pump, vacuum pump, air compressor, air storage tank, water storage tank, and coagulation reaction tank.

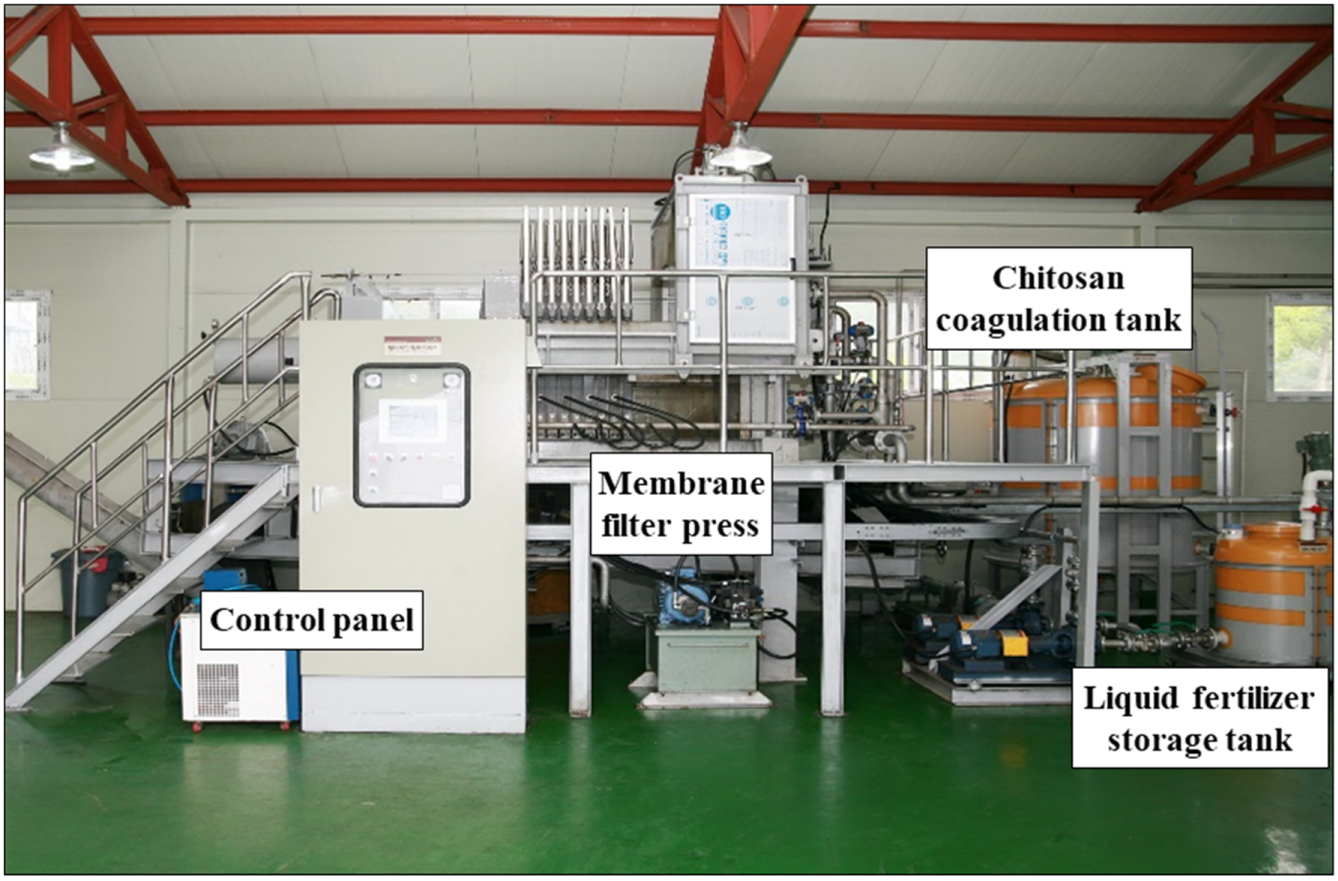


Figure 6. Large-scale membrane filter press system following a scale-up design.

3.2.4. Disinfection Drying Using Microwave Dryer

First, we derived the optimal irradiation height based on the moisture content after drying. The optimal irradiation height is generally proportional to the irradiated area. In typical industrial continuous microwave dryers, irradiation height varies depending on the magnetron arrangement. However, we noted that the width (W) and height (H) were generally fixed and the length (L) increased when the number of magnetrons increased. The optimal W/H ratio calculated from the irradiated area was 1:0.7. In microwave drying, the thickness of the material is an important determinant of drying efficiency. Thinner materials are more conducive to drying but limit throughput, whereas thick materials show lower treatment efficiency. In this study, we explored the maximum thickness of the sample based on the drying efficiency. We observed little difference in the drying efficiency of 1- and 2-cm-thick samples, whereas 3-cm-thick samples had a 1.5- to 2-times lower efficiency. Thus, the maximum thickness of the sample should be $\leq$2 cm.

Based on these experimental findings, we fabricated a large-scale microwave dryer (Figure 7) with a two-tiered structure (top and bottom, to increase site utilization) consisting of a conveyor unit, primary and secondary drying chambers, and a control panel. The dewatered cake produced by the filter press enters the unit through the conveyor (upper right corner) and is moved along by a conveyor belt, during which the dewatered cake is dried by microwave irradiation, and dried protein feedstuff is produced at the bottom on the right side. The drying chamber has a two-layer structure to promote operator safety during microwave drying. The front, back, and sides of the chamber were equipped with windows for the operator to monitor the process in real time. A leveling machine was installed inside the dryer to control the thickness of the dewatered cake placed on the conveyor belt (<2 cm). The conveyor belt was designed with an inverter that enables the modulation of its speed and drying residence time of the sample. The optimum drying residence time was set to 60 min based on a continuous operation experiment with the large-scale microwave dryer.

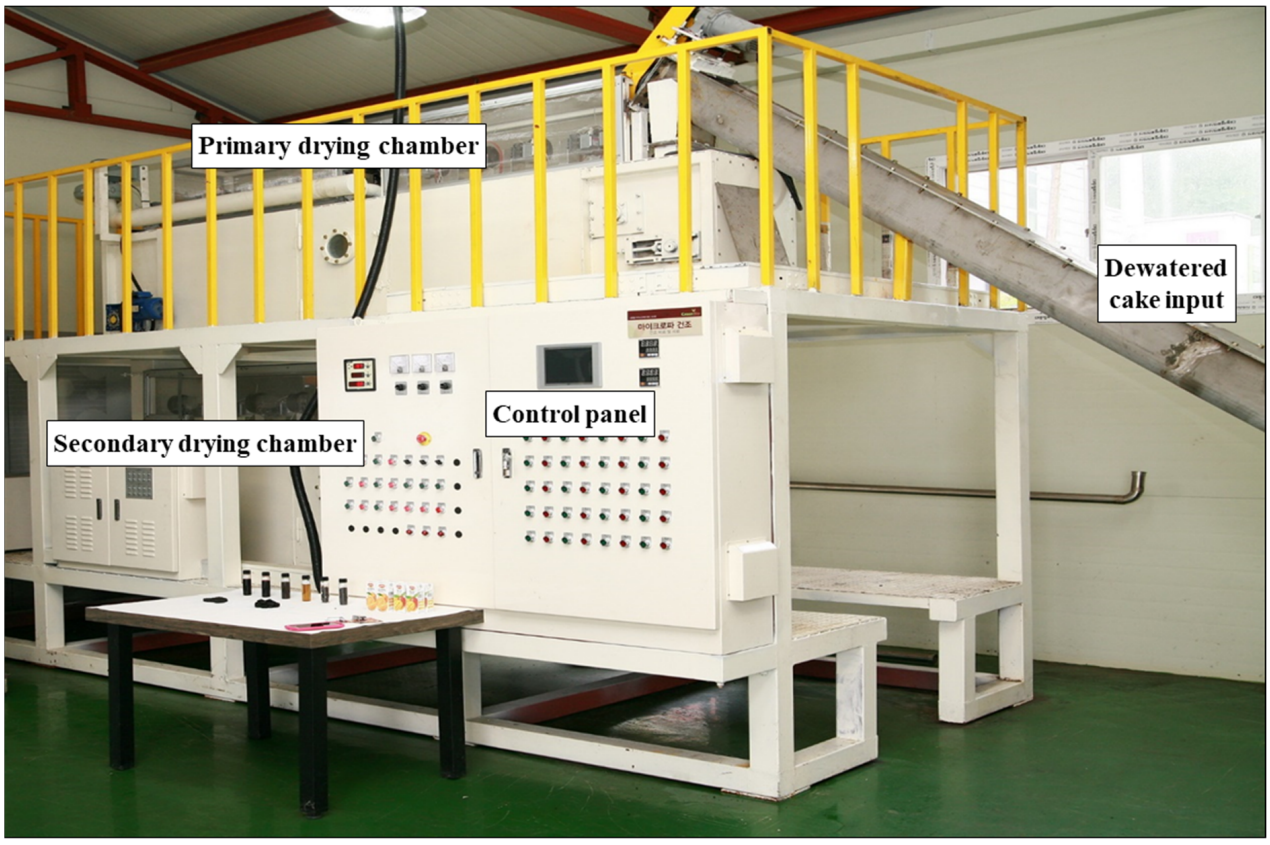


Figure 7. Large-scale microwave dryer system following a scale-up design.

3.3. Integrated Process Configuration and Demonstration

We integrated the ultrasonic solubilization unit, enzymatic degradation reactor, coagulation reaction tank, membrane filter press, and microwave dryer on a single-story factory floor of 23 m in length and 9.2 m in width (Figure 8).



Figure 8. Panoramic view of the large-scale integrated system.

We assessed the performance of the large-scale integrated system using pig blood (Figure 9) sourced from a nearby slaughterhouse. To minimize the degree of coagulation, the blood was collected at dawn or early morning each day and immediately transported to the demonstration site using a refrigerated vehicle. The partially coagulated livestock blood was melted down using a small hand-held agitator, ground in a mill-type grinder, and fed through a 1-mm sieve to remove foreign particles. The crushed and homogenized livestock blood was fed in fixed quantities into the ultrasonic reactor through a transfer pump, and protein solubilization was performed during the ultrasonic pretreatment. After that, the ultrasonically solubilized livestock blood was transferred to the downstream enzyme reactor by a transfer pump, where proteases were added for enzymatic reaction at 60 °C. Next, the enzymatic degradation reaction product was moved to the coagulation tank using a transfer pump, and coagulation was stimulated by mixing with chitosan under continuous agitation. The coagulated product was then transferred to the membrane filter press for filtration and dewatering, following which amino acid liquid fertilizer and dewatered cake were produced. Subsequently, the dewatered cake was subjected to microwave disinfection drying to produce dry protein feedstuff. Notably, the large-scale integrated system operated continuously and semi-automatically.

Figure 10 is the mass balance of the entire system prepared based on continuous operation data. The content of moisture and protein in the livestock blood was 76.11% and 18.23%, respectively. After the ultrasonic solubilization pretreatment and enzymatic degradation reaction, approximately 70% of proteins were converted to amino acids. The membrane filter press produced approximately 61% amino acid liquid fertilizer and approximately 39% dewatered cake based on the total feed volume. The amino acid liquid fertilizer consisted of >20% amino acids, which is 2–8 times higher than that in amino acid liquid fertilizers currently available in South Korea. The content of moisture and protein in the protein feedstuff was <7% and approximately 78%, respectively, which indicates potential for use as high-quality protein feed.

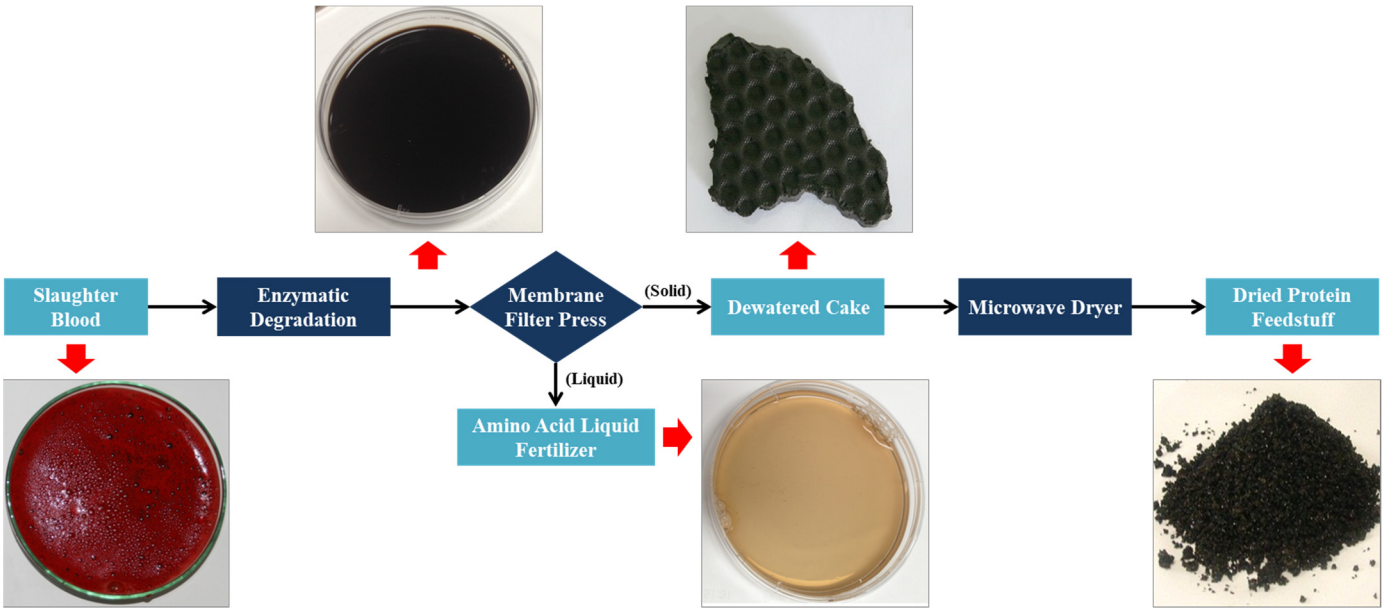


Figure 9. Products from each key unit process.

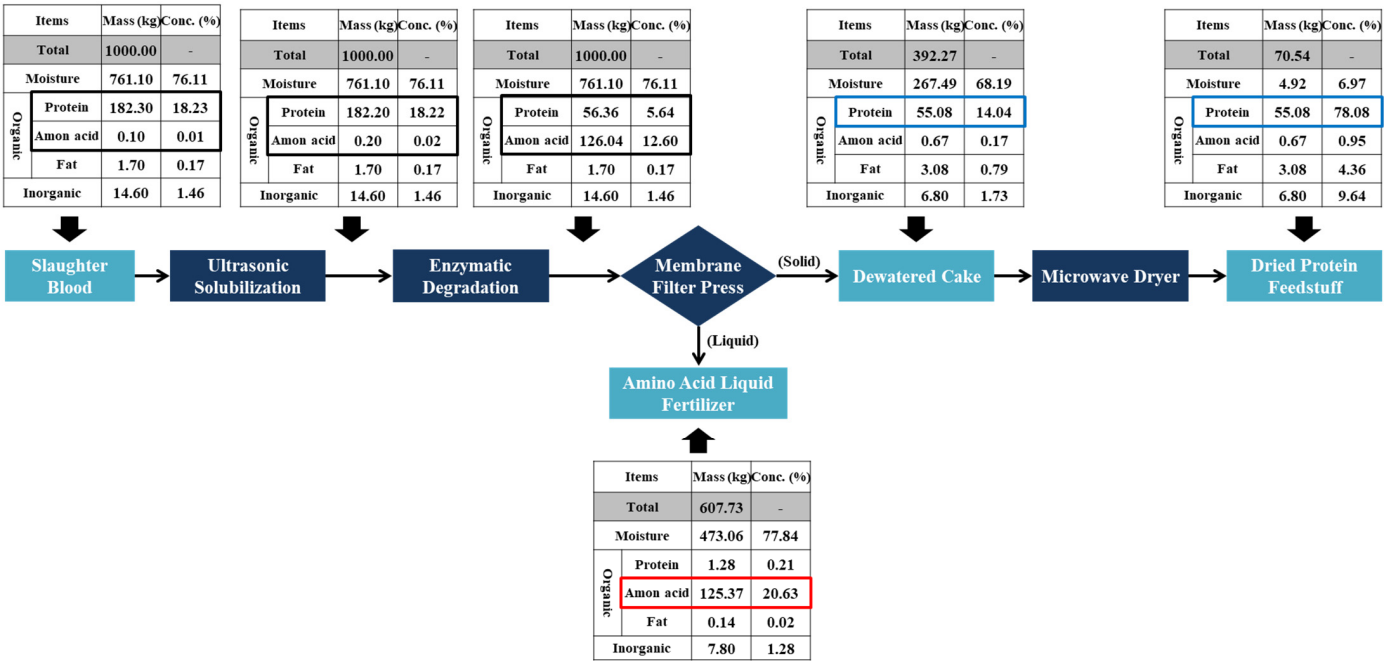


Figure 10. Composition of products for each unit in the large-scale integrated system (mass balance based on a daily throughput of 1000 kg).

3.4. Economic Efficiency of System Operation

The economic analysis was conducted assuming a treatment capacity of 3000 tons/year, with the results presented in Table 2. However, the analysis did not consider the capital cost of installing the system; protein feedstuff production (only considered the production of amino acid liquid fertilizer); bottling, packaging, and distribution costs; and the benefit or cost of raw material (livestock blood). The selling price of amino acid liquid fertilizer was conservatively based on the minimum wholesale price. And the unit price of energy was considered to be approximately 0.0714 € per kWh, which was calculated through the won-euro exchange rate calculation process after applying the average unit price of industrial electricity per kWh in Korea (as of 8 November 2023).

Table 2. Economic feasibility analysis for producing high purity amino-acid liquid fertilizer (based on 3000 tons/year capacity).

Item	Unit	Value
1. BENEFIT	-	-
1.1 Production of high purity amino acid liquid fertilizer	-	-
Yield	ton/year	1820
Wholesale price	€/kg	10–20
Minimum sales revenue	€/year	18,200,000
2. COST	-	-
2.1 Cost of enzymatic degradation reaction	-	-
Initial temperature	°C	5
Reaction temperature	°C	60
Energy consumption	Mcal/year	165,000
Boiler efficiency	%	80
Energy consumption	Mcal/year	206,250
Electricity consumption	kWh/year	239,826
Energy consumption cost of enzymatic degradation reaction	€/year	17,130
Unit price of protease	€/kg	18
Injection rate of protease	% (*w*/*v*)	1.1
Protease input	kg/year	33,000
Protease cost	€/year	594,000
2.2 Cost of ultrasonic pretreatment	-	
Ultrasonic density	W/mL	0.5
Reactor volume	L/batch	200
Ultrasonic time	min	30
Electricity consumption	kWh/year	750,000
Cost of electricity consumption	€/year	53,570
2.3 Cost of membrane filter press	-	-
Electrical capacity of the facility, including accessories	kW	17
Electrical capacity considering efficiency (75%)	kW	22
Daily operating time	h/day	20
Electricity consumption	kWh/year	132,000
Cost of electricity consumption	€/year	9430
Unit price of chitosan	€/kg	30
Injection rate of chitosan	% (*w*/*v*)	0.05
Chitosan input	kg/year	1500
Chitosan cost	€/year	45,000
2.4 Maintenance expense	-	-
Maintenance unit price	€/ton	30
Annual maintenance expense	€/year	90,000
3. BENEFIT–COST	-	-
Annual profit	€/year	17,390,870
Annual profit per ton	€/ton	5812

We estimated an annual profit of 17.4 million euros/year (5812 euros/ton), indicating good economic viability. These figures can only be achieved if the sales channel for the final product—amino acid liquid fertilizer, is acquired.

4. Conclusions

We successfully developed a continuous process equipped to handle five tons of livestock (specifically, pig) blood per day, enabling the simultaneous production of high purity amino acid liquid fertilizer and dried protein feedstuff. The integrated commercial-scale

system consisted of a protein solubilization device that utilizes ultrasound, an enzymatic degradation reactor for amino acid conversion of proteins, a membrane filter press for solid-liquid separation, and a microwave dryer for further dehydration of the solid dewatered cake. These components were based on scale-up designs.

Preliminary experiments suggested that a frequency of 20 kHz, density of 0.5 W/mL, and duration of 10 min/batch were optimal conditions for the ultrasonic pretreatment, which corresponded with a solubilization rate of 97.72%. The optimal enzymatic degradation conditions were determined to be 2 h at 60 °C, comprising a 1-h treatment with Savinase® 1% (*w*/*v*) followed by a 1-h treatment with Flavourzyme® 0.1% (*w*/*v*). Chitosan coagulation was performed to improve solid-liquid separation in the membrane filter press, with the optimal chitosan injection rate determined as 0.05% (*w*/*v*). The optimal dewatered cake thickness and residence time for microwave drying were ≤2 cm and 60 min, respectively, which corresponded with a final W/H ratio of 1:0.7. The ultrasonic solubilization pretreatment and enzymatic degradation reaction converted approximately 70% of proteins in the livestock blood to amino acids. The amino acid liquid fertilizer and dewatered cake accounted for approximately 61% and 39% of the livestock blood, respectively, based on the total feed volume. The amino acid liquid fertilizer contained >20% amino acids. The dry protein feedstuff (produced by microwave drying of the dewatered cake) contained approximately 78% protein. The economic feasibility analysis of the integrated system based on an annual treatment capacity of 3000 tons yielded an annual profit of 17.4 million euros (5812 euros/ton).

This research demonstrates the technical and economic feasibility of producing valuable agricultural resources from discarded livestock blood. It also suggests the potential to mitigate the negative environmental impacts associated with disposing of livestock blood while simultaneously generating useful materials from slaughter-industry byproducts for practical use in the agriculture and livestock industries.

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