Investigation of Low-Cost Amino Acid Production Methods from Abundant Nitrogen-Rich Green Materials in Sri Lanka

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***Abstract*—Amino acid fertilizer is a type of organic fertilizer that is made up of the building blocks of proteins. It is a highly effective fertilizer that can be used to improve soil fertility, plant growth, and crop yield. Amino acid fertilizer is also important for organic farming, as it helps to improve the soil's ability to retain nutrients and water. Therefore, this study was conducted to produce amino acid fertilizer using microbial fermentation to secrete amino acids from abundant nitrogen-rich green materials. The method involved selecting a cost-effective amino acid production approach that utilized anaerobic microorganisms from the soil. Two nitrogen-rich plant species, *Tithonia diversifolia*, and *Gliricidia sepium*, were identified for amino acid production. The process included maceration to break down plant materials, followed by hydrolysis to convert proteins into amino acids. Finally, the mixture was fermented in anaerobic conditions. The study found that the Gliricidia mixture had a higher total N content (700 mg/kg) and amino acid concentration (4.375 g/kg) than the Tithonia mixture (420 mg/kg and 2.625 g/kg, at the end of the experiment (on day 7) respectively). These results suggest that *Gliricidia sepium* is a promising green material for the production of amino acid fertilizers using microbial fermentation. This research has the potential to help Sri Lanka's farmers produce their amino acid fertilizer at a low cost, which could improve crop yields and food security.**

Keywords—Organic fertilizer, amino acid, microbial fermentation

I. Introduction

Proteins are made up of substances called amino acids. Proteins and amino acids are basic components of life. Amino acids are the byproducts of the digestion or breakdown of proteins. A basic amino group (NH_2), an acidic carboxyl group (COOH), and an organic R group (side chain) that is specific to each amino acid make up an amino acid which is an organic molecule [1]. Amino acids are also organic fertilizers and they are usually manufactured by the raw materials of the animal residual body, plant straw, caster bean cake, and vinasse as well [2]. These fertilizers are stress-reducing agents and good nitrogen resources as well. Mostly, they are used in crop production for better results. It contributes to the faster, more efficient absorption of nutrients by the plant. The key benefit of utilizing this is that plants may quickly assimilate the amino acid without the need for any chemical or microbial digestion. Plants immediately utilize amino acids through plant tissues, and the excess breaks down into water-soluble nitrogen is absorbed by the plant roots [3]. Moreover, there is a good possibility to use the waste of amino acid production as a raw material for biogas and biochar production.

Within the last two decades, biotechnical engineering methods for amino acid production have shown major improvements in the agriculture sector. According to the latest statistics, half of the world's population is covered by synthetic nitrogen fertilizers [4]. Normally, organic fertilizer of amino acids is made from animal and plant waste. These contain rich amounts of protein, phosphorus, and potassium. Amino acid fertilizers are the most recent plant nutrition innovations in agricultural production systems. [5]. Amino acid fertilizers are becoming increasingly popular due to their ability to improve crop yields and quality. The global amino acid fertilizer market is expected to grow significantly in the coming years, driven by increasing demand for high-quality food and the need for sustainable agriculture practices. The production of amino acid fertilizers is distributed across various regions of the world, with Asia-Pacific dominating the market. China and India are the major producers in the region [6]. Europe and North America are also significant markets for amino acid fertilizers, with the growing trend towards organic farming and sustainable agriculture practices [7]. Hence, it will provide basic nutrients for crops and update the soil's organic matter. Hence, it is crucial to concentrate on these components and techniques to produce fertilizers using amino acids. Therefore, this study evaluated the potential of using the green materials *Gliricidia sepium* and *Tithonia diversifolia* to produce amino acid fertilizers using microbial fermentation

II. Materials and Method

A. Selection of Amino Acid Production Method and Microorganisms

The methodology commenced with a comprehensive literature review to identify a cost-effective amino acid production method that leveraged nitrogen-rich green materials and biomass-derived intermediates. The chosen method involved laboratory-scale microbial fermentation, utilizing anaerobic microorganisms obtained from soil.

B. Microorganisms Containing Media Selection

The research assumed the presence of fermentative bacteria, specifically *Corynebacterium glutamicum* and *Bacillus spp.* These facultative anaerobic gram-positive bacteria were expected to be present in soil, sewage, vegetables, and fruits [8]. Soil samples were collected from agricultural farmland for the extraction of these microorganisms with the use of a hand glove, spatula, and sterile polythene bag. A spatula was used to remove the overlying earth and collect samples from about 3 cm depth [9]. The microbial culture was separated by diluting the soil sample in sterile normal saline and heated at 80 °C for 10 minutes.

C. Identification of Abundant Nitrogen-Rich Green Materials

After researching nitrogen-rich plant species in Sri Lanka, two primary candidates, *Tithonia diversifolia* (Mexican sunflower) and *Gliricidia sepium*, were identified as nitrogen and potassium-rich green materials suitable for amino acid production. Both plants have high nitrogen contents, typically around 4.2% and 4.7%, on a wet basis respectively [10]. This makes them valuable sources of nitrogen for crops, which is an essential nutrient for plant growth.

D. Maceration and Hydrolysis for Amino Acid Production

i). Maceration Process

The methodology initiated with the maceration process is aimed at breaking down raw materials, primarily plant-based sources rich in proteins, into smaller particles. This physical breakdown increased the surface area, enabling subsequent processes, especially hydrolysis, to access valuable components within the raw materials. The raw materials, *Gliricidia sepium* and *Tithonia diversifolia* (Mexican sunflower leaves) were mechanically crushed using a grinder.

ii). Hydrolysis

After maceration, hydrolysis was the critical step wherein proteins present in the raw materials were broken down into individual amino acids. The addition of water in a 1:2 ratio to the macerated raw materials mixture created an environment conducive to enzyme activity. Hydrolysis experiments were conducted over a 24-hour duration.

iii). Fermentation

The methodology progressed to the fermentation stage, wherein the mixture obtained from the maceration and hydrolysis process was combined with the extracted microorganism culture to initiate the fermentation process. Sealed containers were used to create an anaerobic environment for the fermentation process. Monitoring was essential to determine the endpoint, dependent on the specific microorganisms used and the desired outcome. Samples were collected every 24 hours to facilitate qualitative and quantitative estimations of amino acid production for 7 days. To analyze total nitrogen samples were collected on the 1^{st}, 3^{rd} 5^{th}, and 7^{th} day of the experiment period. Available potassium (K) (wet digestion (HNO_3: $HClO_4$ (9:4)); estimated using atomic absorption spectroscopy), available phosphorous (P) (wet digestion (HNO_3: $HClO_4$ (9:4)); vanadium phosphomolybdate method; estimated using UV-visible spectrophotometer) were analyzed on the last day of the experiment (7^{th} day).

iv). Laboratory Scale Experiments

The methodology involved the utilization of the Kjeldahl method for testing total nitrogen concentration. This technique involved digesting prepared fertilizer samples with a catalyst and sulfuric acid, followed by distillation and titration with sodium hydroxide. For amino acid concentration analysis, the N:P factor (6.25) was employed [11]. This factor, which estimates protein content based on nitrogen analysis, was instrumental in calculating the amino acid concentration. Daily analysis included parameters such as pH (using pH meter Thermo Scientific, model Orion 2 star), electrical conductivity (EC), total dissolved solids (TDS) (EC and TDS meter, Thermo Orient Model 145 A), total solids (TS) (using oven-dried method (using APHA Method 2540-G), and total suspended solids (TSS) using the gravimetric method (using APHA Method 2540-G).

III. Results and Discussion

Two types of treatments were produced under an anaerobic environment using *Gliricidia sepium* and *Tithonia diversifolia* as the raw materials. Only matured leaves were taken for the experiment. When grinding the leaves, it was observed that *Tithonia diversifolia* has a higher moisture content than *Gliricidia sepium,* while *Gliricidia sepium* has a higher fiber content. The soil sample was taken as the medium containing fermentative microorganisms, assuming that this medium contained fermentative bacteria. The soil sample was collected from uncultivated agricultural farmland at the university premises. It was observed that the agricultural farmland had been subjected to a period of heavy rainfall, which resulted in elevated soil moisture levels. The composition of suitable microorganisms in soil samples may change under these conditions.

In this experiment, two mixtures, Gliricidia and Tithonia, were studied over 07 days to assess changes in various parameters. The Gliricidia mixture initially experienced a decrease in pH due to acidogenic reactions. In contrast, the Tithonia mixture maintained a more favorable pH range for agricultural applications throughout the experiment. Both mixtures exhibited increasing electrical conductivity, TDS, and TSS, suggesting the accumulation of ions, metabolites, and microbial biomass as shown in Table 1. The EC of the Tithonia mixture was slightly higher than that of the Gliricidia mixture throughout the experiment. Both mixtures showed a gradual increase in EC values, likely due to the accumulation of ions as a by-product of the fermentation process. On day seven, both mixtures had an EC value of 7,596 mS/cm, indicating a significant increase from their initial values. Similar to EC, the TDS values of both mixtures exhibited fluctuations over the experimental period. On day seven, both samples had TDS values of 3,798 ppm, showing an increase from their initial values. The accumulation of metabolites and waste products from the fermentation process contributed to the increase in TDS.

The TSS content in both mixtures increased significantly by the end of the experiment. The initial and final TSS values of the Gliricidia mixture were 8,000 mg/l and 54,000 mg/l, respectively, while the TSS values of the Tithonia sample changed from 7,000 mg/l initially to 44,000 mg/l at the end. TSS represents solid particles suspended in the liquid phase of the fermentation broth, including microbial cells and undigested organic matter. The increase in TSS is associated with the growth and proliferation of microorganisms during fermentation. The TS content also exhibited continuous fluctuations in both mixtures throughout the experiment, with the Tithonia mixture having slightly higher values. On day seven, the TS values of both mixtures were close, indicating an accumulation of both dissolved and suspended solids, including microbial biomass, cell debris, and undigested organic matter.

TABLE 1. CHARACTERISTICSOOF TREATMENT MIXTURES DURING THE EXPERIMENTAL PERIOD

Sample	pH	EC (ms/cm)	TDS (ppm)	TS (mg/l)	TSS (mg/l)
Tithonia d.	6.99 ±0.16	6851.14 ±898.12	3409.43 ±449.02	20400 ±6485.88	19000 ±12206.56
Gliricidia s.	4.7 ±0.37	6759.71 ±741.22	3380 ±370.53	27057.14 ±4878.13	22000 ±15307.95

The nutrient contents of the two mixtures were dependent on the microbial activity throughout the experimental period. When comparing the initial nutrient contents of both mixtures, the total N content of the Tithonia mixture (210 mg/kg) was higher than the Gliricidia mixture (140 mg/kg) and after that, the total N content of the Gliricidia mixture was increased gradually and had a very high level of nitrogen content (700 mg/l) compared to the Tithonia mixture (420 mg/kg) at the end of the experiment. On the other hand, the Tithonia mixture had a high value of available K (1,641.4 mg/kg) compared to the available K (1,521.9 mg/kg) of the Gliricidia mixture. The Gliricidia mixture had a high value of available P (56 mg/kg) compared to the available P (44 mg/kg) of the Tithonia mixture.

In the Gliricidia mixture, the total N content was 700 mg/kg and the amino acid concentration was 4.375 g/kg on the 7th day which was the maximum reported value. In the Tithonia mixture, the total N content was 420 mg/kg and the amino acid concentration was 2.625 g/kg on the 6th day and the 7th day which were the maximum reported values. It can be due to high microbial population and activities towards the middle of the experiment and then decreasing microbial activities with gradual death of microbes due to lack of substrate. The rapid increase in total N level and the amino acid concentration on the 7th day could be attributed to the increase in the growth of microorganisms along with the gradual increase in pH to a favorable value. Within seven days, total N content and the amino acid concentration of the Gliricidia mixture had a distinguishable increase compared to the Tithonia mixture.

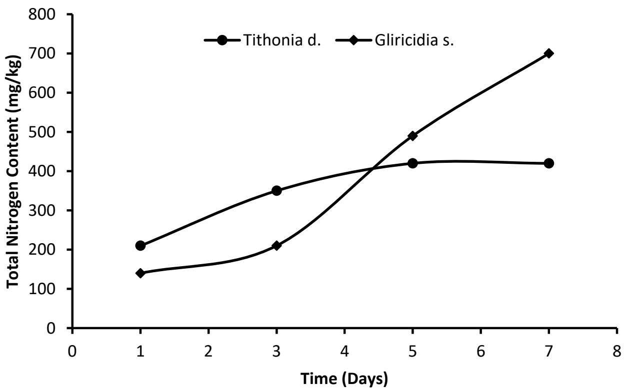

Fig. .1 Variations of total nitrogen concentration of samples with time

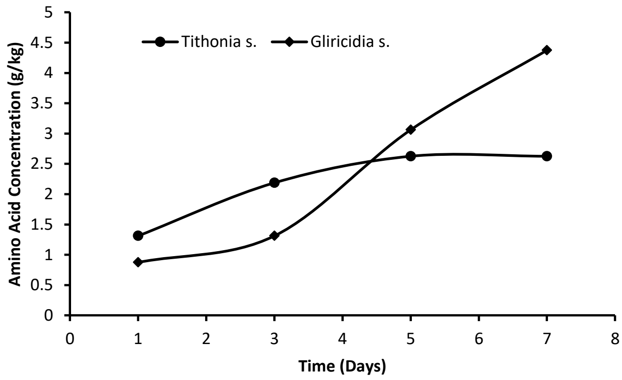

Fig. 2. Variations of amino acid concentration of samples with time

To further enhance this research and maximize amino acids and nitrogen concentration, several strategies can be employed. Implementing a gyratory shaker during the production process to improve the contact between microorganisms and plant materials can significantly enhance amino acid production efficiency. The use of a soil sample from the topsoil of a forest may also improve the efficiency of amino acid production, as this type of soil is likely to contain more effective microorganisms.

The potential applications of this fermented mixture with amino acids are extensive. They can be effectively combined with biochar to produce biochar biocatalysts for organic fertilizer production [14, 15] creating enriched soil conditioners that enhance nutrient retention and promote sustainable agriculture [16]. Also, this type of liquid fertilizer can be easily incorporated into drip irrigation systems. This method ensures a consistent and precise delivery of nutrients to the root zone, promoting even growth and nutrient uptake by plants, and can be applied directly to the root zone by drenching the soil. It ensures that nutrients are delivered to the root system, where they can be readily absorbed. When applied correctly, it can reduce the risk of nutrient runoff and leaching compared to granular fertilizers. This can help minimize the environmental impact on water bodies. Furthermore, as additives in composting with other organic mixtures, these amino acid fertilizers can elevate the overall quality of compost, fostering healthier soil and increased crop yields. Proper storage is crucial for maintaining the quality and effectiveness of amino acid fertilizer. To prevent degradation and contamination, the fertilizer should be stored in compatible, non-reactive, and corrosion-resistant containers with tightly sealed lids [12]. A cool (4 °C is preferred) dark place is ideal for storage to protect the fertilizer from direct sunlight and high temperatures, which prevents degradation reactions [13]. It is vital to assess the economic feasibility of the fertilizer production process, aligning with sustainable practices by repurposing garden waste and utilizing agricultural land resources without incurring additional financial demands.

IV. CONCLUSION

This research has delved into the potential of utilizing *Gliricidia sepium* and *Tithonia diversifolia* as green materials for the production of amino acid fertilizers through microbial fermentation. The findings indicate the superior performance of *Gliricidia sepium* over *Tithonia diversifolia*, with significantly higher total nitrogen content and amino acid concentration. The observation that the liquid sample contained suspends on day 7 suggests that the fermentation process was still ongoing. The fermentation conditions, such as temperature, pH, and nutrient availability, can have a significant impact on the production of amino acids and nitrogen. By optimizing these conditions, it is possible to further increase the concentration of these nutrients in the fertilizer. The potential applications for these amino acid fertilizers are diverse and far-reaching. They can be used to improve crop yields in a variety of settings, from smallholder farms in developing countries to large-scale agricultural operations. With ongoing adjustments and experimentation, there is substantial room for further refinement, leading to the creation of even more efficient and effective amino acid fertilizers.

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