Chemical Engineering Journal 302 (2016) 146–154

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

# An integrated electrodialysis-biocatalysis-spray-drying process for efficient recycling of keratin acid hydrolysis industrial wastewater

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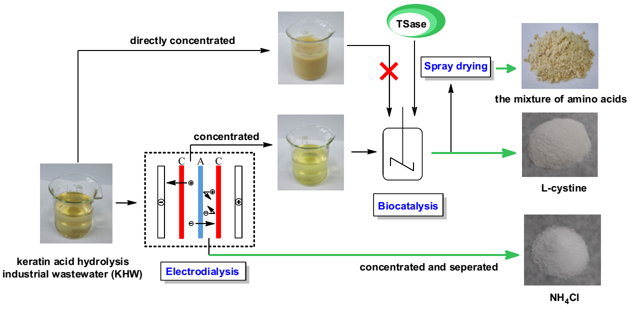
## HIGHLIGHTS

- Keratin acid hydrolysis wastewater was fully recycled by a novel integrated process.
- The ionic species in wastewater could be efficiently removed by electrodialysis.
- The treated wastewater increased the production of L-cysteine five folds by TSase.
- The conversion fluid was retrieved by spray-drying as fertilizer or feed additives.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

*Article history:*
Received 11 March 2016
Received in revised form 10 May 2016
Accepted 11 May 2016
Available online 12 May 2016

*Keywords:*
Keratin acid hydrolysis wastewater
Electrodialysis
L-Cysteine
Tryptophan synthase
Spray-drying

## ABSTRACT

In this work, a novel and efficient electrodialysis-biocatalysis-spray-drying integrated process was developed for the complete recycling of keratin acid hydrolysis industrial wastewater. The ionic species including ammonium chloride, L-aspartic acid, and L-glutamic acid were removed from the wastewater by electrodialysis, and their removal efficiencies were 99.96%, 91.62%, and 84.58%, respectively. Compared with the formation of a light brown paste of the original wastewater, the wastewater treated with electrodialysis was concentrated to a high concentration of L-serine solution (120 g/L). Although L-serine from the wastewater could be converted into L-cysteine, L-tryptophan or S-phenyl-L-cysteine by recombinant tryptophan synthase, the production of L-cysteine was the optimal route to efficiently recycle the wastewater. The efficiency of production of L-cysteine increased approximately five times via electrodialysis treatment. In the scale-up study, the molar conversion rate of L-serine reached 96%, and L-cysteine was purified from KHW as its oxidized form, L-cystine, with a total yield of 90%. After L-cystine was collected, the conversion fluid was dried by spray-drying to yield a mixture of amino acids, which could be used as feed additives or fertilizer. In comparison with directly draining or concentrating the original wastewater to obtain the low-value mixture, this study has for the first time provided an effective strategy for completely recycling keratin acid hydrolysis industrial wastewater to produce high-value products including L-cysteine and a mixture of amino acids.

## 1. Introduction

In recent years, the extraction of amino acids from protein acid hydrolysates has become one of the most prevailing methods for the industrial production of amino acids. As a result, more than 180,000 tons of keratin acid hydrolysis industrial wastewater

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http://dx.doi.org/10.1016/j.cej.2016.05.046
1385-8947/

(KHW) is produced in amino acids industry annually in China, accounting for 60% of global KHW production [1–3]. Therefore, it is necessary to recycle the wastewater efficiently. At present, KHW is drained or collected as fertilizer by concentrating KHW. However, the crude contains a significant content of ammonium chloride, which directly restricts its application as fertilizer and makes it low-valued. On the other hand, the direct drainage of KHW causes serious environmental problems. In fact, the wastewater is still a precious resource of amino acids. The separation of ammonium chloride in wastewater would enable the recycle of wastewater to manufacture high value-added products. The treated KHW could be simply dried by spray-drying to afford a mixture of amino acids, which could be more suitable for fertilizer, even for feed additives, thus alleviating the environmental issues caused by direct drainage and adding additional value to the products.

Over the past decade, to enhance the value of by-products and reduce the pollution to the environment, many research groups have developed various methods to recycle some high value-added products from organic wastes [4–14]. Among them, biological methods are more environmentally friendly in recycling the wastewater. Although the hair acid hydrolysis wastewater (HHW) could be used as the raw material to synthesize L-tryptophan and S-phenyl-L-cysteine by tryptophan synthase (TSase, EC 4.2.1.20) [1–2], the production efficiency is low due to the low concentration of substrate in wastewater, in which the concentration of L-serine is only about 20 g/L. If KHW is utilized to synthesize the high valued product enzymatically (*e.g.* L-cysteine, Scheme 1), the same problem will arise since the concentration of L-serine in KHW is approximate 20 g/L too. Evidently, it is very uneconomical if the wastewater is directly used to synthesize L-tryptophan, S-phenyl-L-cysteine or L-cysteine. According to the literature, the optimal content of pure L-serine as a substrate of TSase to synthesize L-cysteine ranges from 1 to 25 wt% [15]. It is reasonable that increasing the content of L-serine in KHW could improve the production efficiency. However, KHW could become a thickened liquid simply by evaporation at high temperatures (85–95 °C) and turn into a light brown paste when cooled down to room temperature, which is not a proper substrate of TSase. Studies also illustrated that ammonium chloride, the major salt in KHW, and the amino acid complex could be the primary factors responsible for the formation of a sticky liquid. Therefore, the removal of ammonium chloride is imperative to obtain KHW solution with a high concentration of L-serine.

Electrodialysis is an electrochemical separation process with cation and anion exchange membranes employing an electric potential as the driving force, which has been considered as an effective method for the purification of wastewater containing ionic species [12,16–18]. Therefore, electrodialysis was chosen to remove the excessive ions in KHW because of their ionization properties. In this work, electrodialysis was applied to purify KHW by separation of ammonium chloride from KHW. Subsequently, the treated KHW was concentrated and used as the substrate of TSase to produce the high-value product (*e.g.* L-cysteine).

L-Cysteine is an important amino acid that is widely used in pharmaceutical, food, forage, cosmetics, and detergent industries [19–20]. The development of an economical method for the production of high-purity L-cysteine has thus become an increasing need for industrial manufacture. Although L-Cysteine could be efficiently synthesized by TSase with L-serine and sodium hydrosulfide (NaHS) as substrates (Scheme 1) [21], the cost of L-serine accounts for a significant portion of the cost in the synthesis of L-cysteine. If L-serine originated form KHW and more economical TSase recombinant whole cells instead of the isolated enzyme [22] could be used to prepare L-cysteine, it would substantially reduce the cost and stimulate the development of recycling the wastewater.

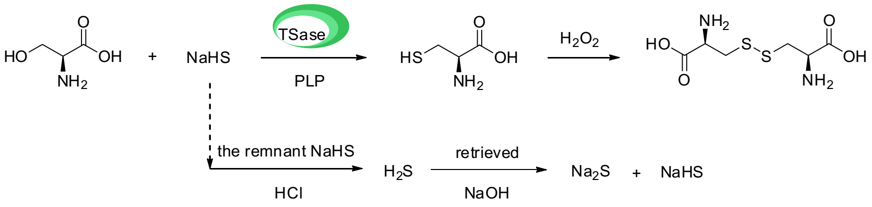
After L-cysteine was separated from the conversion fluid and excessive sodium hydrosulfide (NaHS) was recovered, the resulting KHW was concentrated by spray-drying. Spray-drying consists of the atomization of a solution or liquid suspension into tiny drops and drying in a stream of hot air to produce fine powders [23]. Spray-drying has been successfully applied in food and pharmaceutical industries because this process is relatively flexible, simple, low-cost, and the powder is produced with high quality and stability [24].

In the present study, the potential of fully recycling KHW was investigated. It has been shown that KHW could be effectively purified by electrodialysis in which both ammonium chloride and some acidic amino acids (*e.g.* L-aspartic acid, L-glutamic acid) could be concurrently removed from KHW. The KHW treated with electrodialysis could be concentrated by rotary evaporation to afford a light yellow liquid in which the concentration of L-serine would increase by six times compared with the original KHW. To fully recycle KHW, we carried out the optimal enzymatic synthesis of L-cysteine and evaporated KHW by spray-drying to obtain the light-brown powder, which could be a better feed additives or fertilizer. Table 1 shows the comparison result of reactions catalyzed by TSase whole cells in different reaction systems. Compared with the previous studies, this study has provided an effective strategy for fully recycling KHW to produce the high-value product for the first time. Furthermore, we have optimized electrodialysis time for KHW and studied the effects of various reaction parameters on the enzymatic reactions under aqueous conditions. Meanwhile, bioconversion efficiency and product yield were determined under the optimal conditions.

## 2. Materials and methods

### 2.1. Materials

KHW used as a raw material was provided by Shine Star (Hubei) Biological Engineering Co. Ltd. (Hubei, China). The ingredients of KHW depend on the species of initial keratin used in amino acid production, but most KHW contained ammonium chloride, pigments, suspended solids, and approximate nine amino acids as shown in Table 2. Lactose, pyridoxal 5'-phosphate (PLP), and Triton X-100 were purchased from Sigma (St. Louis, Mo., USA). All other chemicals and reagents used in this work were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd (Shang-



**Scheme 1.** The reaction routes involved in the present study. TSase = tryptophan synthase from *E. coli* K-12 MG1655, PLP = pyridoxal phosphate.