

can increase costs. Further, one needs to remove the counter ions of the salts so as to yield free acids, and one needs to remove and dispose of any resulting waste and by-products. For instance, calcium salts of C_4 diacids have a very low solubility in aqueous broth solutions (typically less than 3 g/liter at room temperature), and are not suitable for many applications for which a free acid species is needed, such as chemical conversion to derivative products. Therefore, the calcium salt is typically dissolved in sulfuric acid, forming insoluble calcium sulfate, which can readily be separated from the free diacid. Calcium sulfate is a product having few commercial applications, and accordingly is typically discarded as a solid waste in landfills or other solid waste disposal sites. All of these individual operational units contribute to the overall costs of the process.

The production costs for the bio-based carboxylic acids have as a result been too high for bio-based production to be cost-competitive with petrochemical production regimes. (See e.g., Janes McKinlay et al., "Prospects for a Bio-based Succinate Industry," *APPL. MICROBIOL. BIOTECHNOL.*, (2007) 76:727-740; incorporated herein by reference.) For example, with most commercially viable succinate producing microorganisms described in the literature, one needs to neutralize the fermentation broth to maintain an appropriate pH for maximum growth, conversion and productivity. Typically, the pH of the fermentation broth is maintained at or near a pH of 7 by introduction of ammonium hydroxide or other base into the broth, thereby converting the di-acid into the corresponding di-acid salt. About 60% of the total production costs are generated by downstream processing, e.g., the isolation and purification of the product in the fermentation broth.

Over the years, various other approaches have been proposed to isolate the di-acids. These techniques have involved using ultra-filtration, precipitation with calcium hydroxide or ammonia, electrodialysis, liquid-liquid extraction, sorption and ion exchange chromatography. (See, Tanja Kurzrock et al., "Recovery of Succinic Acid from Fermentation Broth," *Review, BIOTECHNOLOGY LETTER*, (2010) 32:331-339; incorporated herein by reference.) Alternative approaches that some have proposed include operating a fermentation reactor at low pH, which functionally would be similar to operating the fermentation with minimum level of salts. (See, e.g., Carol A. Roa Engel et al., "Development of a Low-pH Fermentation Strategy for Fumaric Acid Production by *Rhizopus oryzae*," *ENZYME AND MICROBIAL TECHNOLOGY*, Vol. 48, Issue 1, pp. 39-47, 5 Jan. 2011, incorporated herein by reference.)

For example, FIG. 1 shows a schematic diagram of a known process for extracting organic acids from a fermentation broth. Glucose, corn steep liquor, or other sugars, and $CaCO_3$ are introduced into a fermentation reactor 1 and subjected to microbial fermentation 2. A fermentation broth liquid containing a mixture of organic acids and other by-products 3 is extracted and filtered 4. The broth is neutralized 5 with a strong acid, such as H_2SO_4 , which generates $CaSO_4$. The reaction mixture is then filtered 6 to remove cell mass and the $CaSO_4$ 7, which is waste that cannot be recycled; hence, it is disposed of in landfill or employed for gypsum-using applications. The remaining organic acids, glycerol, and other by-products 8 can be recovered and fed back into the fermentation reactor as a carbon source, such as described in U.S. Pat. No. 8,183,022, the content of which is incorporated herein by reference. The products can be separated by various techniques, such as crystallization or ion exchange 9. The organic acids can be purified 10, for example, over a carbon bed.

An alternative approach some have described involves the synthesis of alkyl monoesters by direct esterification of alkali metal salts of carboxylic acids, such as calcium lactate, sodium acetate, sodium benzoate, and sodium salicylate, using carbon dioxide and an alcohol as a way of making bio-based chemicals in an environmentally friendly manner (see, Prashant P. Barve, et al., "Preparation of Pure Methyl Esters From Corresponding Alkali Metal Salt of Carboxylic Acids Using Carbon Dioxide and Methanol" *IND. ENG. CHEM. RES.*, 15 Sep. 2011.). The esterification process, however, has a limited application and do not describe the recovery of polycarboxylic acids.

Although these techniques have had some success, they are not able to provide a direct route by which fermentation-derived dicarboxylic or polycarboxylic acids can be recovered in a simple, cost-efficient process from a fermentation broth. Rather, these fermentation techniques often involve the need to go through several different steps to prepare the carboxylic acids in fermentation broth for chemical transformation and to convert the raw acids to useful compounds.

To reduce waste and costs associated with generating free carboxylic acids and to improve the recovery yield, a need exists for a better, more direct method of recovering a variety of carboxylic acids, such as malic or succinic acid, and which can provide a successful route to simplify downstream chemical conversions from a biologically-derived feedstock. Such a streamlined, green process would be a welcome innovation.

SUMMARY OF THE INVENTION

The present invention describes, in part, a process for recovering and using carboxylic acids from a fermentation broth by converting a carboxylic acid to one or more of its corresponding esters (i.e., monoester, diester, or triester) in a relatively efficient and cost effective manner. In particular, the present process involves obtaining a fermentation broth, from which cell mass and insoluble compounds have been either removed or not, containing at least one free carboxylic acid, or a mixture of carboxylic acids, or at least one free carboxylic acid and an associated alkali or alkaline earth metal salts of the carboxylic acid (e.g., sodium, potassium, or magnesium salts); drying the raw or clarified fermentation broth containing free carboxylic acid into a powder; and reacting the carboxylic acid in the powder with an alcohol under a CO_2 atmosphere in the substantial absence of any other acid catalyst, at a reaction temperature or pressure corresponding to supercritical, critical or near critical conditions for at least the alcohol or CO_2 , to synthesize the corresponding ester or esters from the carboxylic acid in the powder. In subsequent steps, the esters can be converted back to their corresponding free acid form. One may recycle the synthesis by-products directly back into the original or a new fermentation broth.

The esterification reaction temperature is between about $150^\circ C.$ and about $250^\circ C.$, and the operational reaction pressure is between about 400 psi and about 3,000 psi (gauge). Depending on the desired results, the reaction can be run for about 4 hours, up to about 12 hours.

In another aspect the present invention pertains to a method for esterifying a polycarboxylic acid derived from fermentation. The esterification method involves: providing a solution of one or more free carboxylic acids from a fermentation broth and reacting the free carboxylic acids with an alcohol in a CO_2 atmosphere without the presence of any other acid catalyst; and selecting an operational reaction temperature or reaction pressure corresponding to supercritical