Final Scientific/Technical Report

1. Identify the DOE award number; name of recipient; project title; name of project director/principal investigator; and consortium/teaming members.

   DOE award # (NREL): No. DE-AC36-08GO28308

   Name of recipient: ORNL is lead lab, ANL and NREL are team members

   Project Title: ***Novel Approaches to the Isolation of Glucaric Acid from Fermentation Broth***

   Principle Investigator: Aimee Church

   Consortium team members: ANL (Yupo Lin, yplin@anl.gov; Phil Laible, laible@anl.gov), ORNL (Aimee Church, lum1@ornl.gov), NREL (Eric Karp, eric.karp@nrel.gov)

2. Display prominently on the cover of the report any authorized distribution limitation notices, such as patentable material or protected data. Reports delivered without such notices may be deemed to have been furnished with unlimited rights, and the Government assumes no liability for the disclosure, use or reproduction of such reports.

   NREL patent *- U.S. Patent App. No. 63/273,234 filed on October 22, 2021*

   NREL Publication *– Accepted for publication in Green chemistry (accepted December 2021)*

   Separation of Bio-Based Glucaric Acid via Antisolvent Crystallization and Azeotropic Drying‡

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3. Provide an executive summary, which includes a discussion of 1) how the research adds to the understanding of the area investigated; 2) the technical effectiveness and economic feasibility of the methods or techniques investigated or demonstrated; or 3) how the project is otherwise of benefit to the public. The discussion should be a minimum of one paragraph and written in terms understandable by an educated layman.

Glucaric acid (GA) is regarded as a top-value added compound from biomass, however, due to prevalent lactonization, the recovery of purified glucaric acid is challenging. Accordingly, an efficient method for glucaric acid separation, especially its diacid form, is necessary to facilitate its valorization. Kalion has successfully demonstrated a fermentation route using glucose as the feedstock. This game-changing technology allows Kalion to economically produce large-scale quantities of high-purity glucaric acid products. Kalion has also developed a novel downstream processing strategy for isolating and purifying glucaric acid forms, including the free acid, monoammonium glucarate, and monopotassium glucarate (KGA). Despite the low solubility of these products, significant water removal is needed prior to crystallization, requiring energy intensive evaporation or reverse osmosis. Here, Kalion works with Separations Consortium to develop novel, low-cost means of water removal in Kalion's glucarate/glucaric acid purification process. This CRADA project with national labs (ORNL, ANL, and NREL) evaluated various technologies developed by the Separations Consortium as applied to glucaric acid purification, intended to generate a scalable, cost-effective, energy-efficient process, which includes (1) Membranes, Fabrication, and Process Evaluation (ORNL); (2) Resin Water Electrodionization (ANL), (3) Functionalized Nano-sorbents (ANL), and (4) Acidification and antisolvent crystallization In situ Product Recovery (ISPR) system in the Bioproduct Separations laboratory (NREL).

4. Provide a comparison of the actual accomplishments with the goals and objectives of the project. Where applicable, address any comparisons of actual results to programmatic technical barriers and milestones.

   The overall goal of the project was to develop low cost means to remove water in Kalion's monopotassium glucarate process and glucaric acid process

   ORNL - *The key technology advantage of the ORNL process is the use of nano porous membranes, that have super-hydrophilic or super-hydrophobic functional groups coating the surface to selectively remove water and sugar from the organic acid. This nanotechnology allows pore size to be larger than traditional membranes without sacrificing selectivity, thus overcoming the trade-off between selectivity and flux. (https://doi.org/10.1016/j.seppur.2019.116312). In this work, we have tested the application of membranes pervaporation to the concentration of fermentation broth from Kalion's glucaric acid process. First, the membranes were synthesized and tested for their ability to separate water from glucaric acid in a pressure-driven batch process. The separation showed high selectivity and flux: permeate was collected and only contain 0.1% of glucaric acid, with high flux~1 LMH. The second stage membrane was able to separate sugars and glucaric acid successfully. Finally, we tested the durability of the membranes by using integrated membranes that will be able to run >2 L fermentation broths for >30 h, and then discussed with Kalion about the scale-up possibility.*

ANL (RW-EDI) *– Over the course of project period, Argonne have conducted separation and capture of glucaric acid from Kalion's fermentation broth using Argonne's proprietary electrochemical resin wafer separation technology, RW-EDI. Use surrogate and actual fermentation broth from Kalion, Argonne have concluded the experimental evaluations and validation of processing costs and final titers of the capture glucaric acid and their organic salt forms respectively. Preliminary TEA were performed to provide a quick economic assessment of the separation technologies. The TEA shows >98% glucarate capture from the broth, the processing cost is as low as $0.14/kg of captured glucarate salt in 60 wt.% titers. >50% of the Glucaric acid capture from the fermentation with pure acid form in a processing cost of $0.52/kg of pure glucaric acid and >99% purity of 52 wt.% captured pure glucaric acid titer. In both the extractions of glucarate and glucaric acid from the broth, there was less than 0.1% of sugar loss from the broth. It provides a strong likelihood to establish a continuous fermentation by integrating the fermentation and the electrochemical extraction system with an in-situ production capture. The robustness and simplicity of using electric energy to directly capture carboxylic acid enabling a commercial applications of cost-competitive biobased organic acids production for chemical industrial.*

We tested the energy efficiency, separation ratios and processing cost using an electrochemical membrane separations technology to extract k-glucarate from fermentation broth into either glucarate or pure glucaric acid forms. The glucarates were selectively captured from the broth. With different ion-exchange membranes configurations, the glucarates were simultaneously purified and concentrated into high > 50 wt.% of K-organic salt or organic acid. >98% glucarate was extracted from the broth in salt form.

ANL (Functionalized Nano-sorbents) *– In the laboratory of Philip Laible, ANL developed a low cost, passive process to remove glucaric acid from complex fermentation broths. Currently, many methods are in use to separate high-value bioproducts, but approach recoveries using active and more energy intensive methods, including centrifugation, multi-phase extractive fermentation, pH cycling, and/or heating – all ultimately leading to increased costs. Through the use of nano-structured adsorbent based upon patented xerogel technology, this process minimizes energies and costs, especially as these advanced materials can be cycled through recovery efforts for tens (to hundreds) of times before discarding. Additionally, as the advanced materials passively and selectively adsorb glucaric acid or the salt forms of the product, the complex fermentation broth can simply be passed over the xerogel. Then, glucaric acid can be recovered from the material surface in high yield and purity using moderate compression forces (on the order of 20 psi). This process effectively increased the concentration of glucaric acid 7-10 fold from a series of experiments that surveyed bioreactor streams that ranged from aqueous solutions to spent fermentation broths.*

The synthesized nano-sorbents that specifically capture glucaric acid (GA) from complex media with minimal water co-adsorption utilized two different recovery strategies. Recoveries either used surface coating of carboxylic acid scavengers or direct synthesis of nano-sorbents incorporating tertiary and quaternary amines. Unfortunately, this latter strategy – synthesizing nano-sorbents with tertiary/quaternary amine silane precursors – proved to be unsuccessful as polymerization reactions proceeded too quickly causing inhomogenous materials or failed at the catalysis stage. Therefore, successes studied in greatest detail were those derived from nano-sorbents that displayed scavengers on their surface. Three different scavengers, trioctylphosphine oxide (TOPO), trioctylamine (TOA), and Cyanex923 which is a combination of several trialklyphosphine oxides, were studied in detail. Of these three, ~ 20% glucaric acid from a 30g/L glucaric acid solution was recovered using nano-sorbents with TOA or Cyanex923 bound. However, Cyanex923-containing materials adsorbed water from control samples in the absence of glucaric acid and may prove problematic in bioreactor implementation at early phases of production. Thus, TOA proved to be the most promising of the three scavengers examined (with greater recoveries for acid as compared to salt forms of glucaric-acid products.

***NREL** – we developed a robust separation process that produces glucaric acid crystals from fermentation broth. This process first recovers purified monopotassium glucarate from broth and then recovers purified glucaric acid through acidification and antisolvent crystallization. Isopropanol was found to be an effective antisolvent reducing the solubility of glucaric acid while concomitantly forming an azeotrope with water. This allows solvent removal at low temperature through azeotropic drying, which avoids lactonization, and thus prevents impurities in the resulting crystals. Overall, this process was found to separate monopotassium glucarate and glucaric acid with a recovery yield of >99.9 % and 71 % at purities of c.a. 95.6 and 98.3 %, respectively. Process modeling demonstrated the ability to recycle the antisolvents IPA and acetone with >99 % recovery and determined the energy input to be ~20 MJ/kg for isolation of monopotassium glucarate and 714 MJ/kg for glucaric acid (0.06 M). The approach detailed in this work is likely applicable to the separation of other highly oxygenated bio-carboxylic acids (e.g., mevalonic acid) from fermentation broths, as well as to their recovery from abiotic reaction solutions. We developed two methods based on antisolvent crystallization to recover crystalized monopotassium glucarate and glucaric acid from fermentation broth. (1) recovers K-glucarate from fermentation broth using acetone as the antisolvent. Essentially quantitative yield was achieved with 95.6% purity on the product. (2) K-glucarate was cation exchanged and then crystallized using IPA as the antisolvent. Yields of 71% and purities of 98.3% were achieved with glucaric acid. Previously there was no available method to produce purified glucaric acid due to its tendency to lactonize during solvent evaporation. NREL filed a patent on this process and is negotiation with Kalion to license it.*

5. Summarize project activities for the entire period of funding, including original hypotheses, approaches used, problems encountered and departure from planned methodology, and an assessment of their impact on the project results. Include, if

applicable, facts, figures, analyses, and assumptions used during the life of the project to support the conclusions.

NREL - To address the need for optimized downstream processing routes for GA, this work developed a scalable, environmentally friendly, and economically feasible antisolvent separation process for the recovery of GA and its salts from fermentation broth. Antisolvent crystallization involves combining the product solution with another solvent in which the product is only slightly soluble. This significantly reduces the solubility of the product in that solution, allowing it to be recovered as a precipitate. One notable feature is that our process uses antisolvents that are Generally Recognized as Safe (GRAS).

The separation processes are depicted in **Figure 1**. First, dipotassium glucarate (K_2GA) is produced via fermentation at a neutral pH to generate a broth. Solid KGA is then recovered from the broth by employing 1) pH-adjustment from 7 to 3.5 to generate KGA, 2) antisolvent crystallization of KGA using acetone at an acetone-to-water mass ratio of 1 to 2.95, 3) KGA product filtration, and finally 4) acetone antisolvent recycling via distillation of the supernatant. Next, crystalline GA is produced from the purified KGA via another antisolvent crystallization process, which consists of the following steps: 1) cation exchange for acidification and K^+ removal, 2) isopropanol (IPA) antisolvent crystallization of GA, 3) GA crystal recovery by azeotropic drying, and 4) IPA antisolvent recycling. The physicochemical and thermodynamic properties of the purified KGA and GA products were analyzed and used to develop Aspen Plus models for solvent recovery, which enables the calculation of the energy input on the downstream process. Compared to the ACN-water system that was reported in the literature prior and did not work, the IPA system reduces the antisolvent amount by 2.1 times. The antisolvent crystallization process proposed in this work could also be applicable to the purification of other oxidation products from glucose, such as gluconic acid and mevalonic acid.

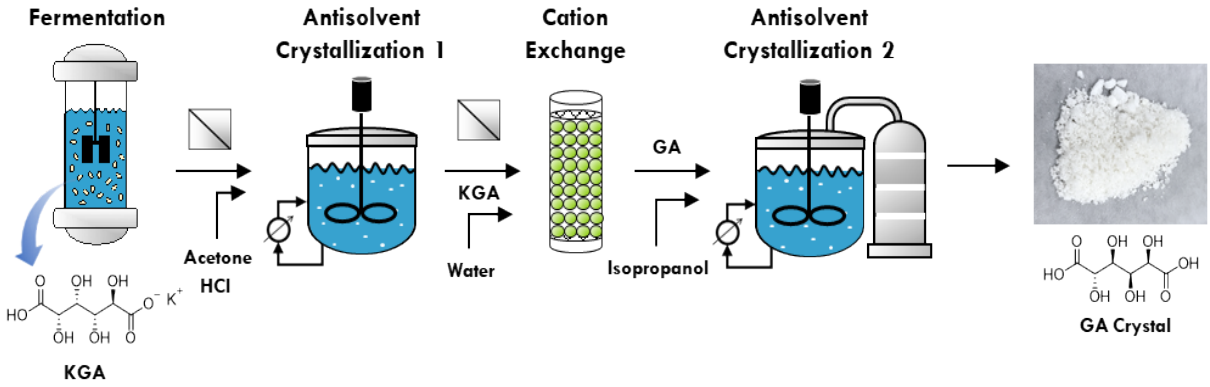

Figure 1: Process flow diagram for producing KGA and GA crystals. HCl was added into Antisolvent Crystallization 1 process for pH control. Acetone and isopropanol were used as antisolvent for KGA in Antisolvent Crystallization 1 and for GA in Antisolvent Crystallization 2

ANL (RW-EDI) – Different ion-exchange resin wafer materials were fabricated and used to optimize the energy efficiency and separation productivity of glucarate separation. Figures 2 and 3 show the RW-EDI membrane configurations and the glucarate extraction performance from actual fermentation broth to produce pure glucaric acid and k-glucarate, respectively.

RW-EDI technology provides a one-step process that enables selective extraction, acidification and concentration of the glucarate into glucaric acid without additional unit operations or acidification chemicals. Acidification was achieved by electrical water splitting to provide the proton (as shown in Fig. 2, the low pH in the capture effluent of RW-EDI device. Fig.3 shows the K-GA extraction, purification and dewatering from the fermentation broth. The low energy consumption and high productivity of separation made the processing cost very competitive. Table 1 lists a preliminary TEA of these two processes. At 50% capture, the pure glucaric acid can be produced at $0.52 /kg of GA. At 98 % capture, the process cost of >99% pure, 60 wt.% K-GA titer was only $0.14/kg K-GA. Figure 4 shows the capture GA product purity, >99.9% of sugar in the fermentation broth was still in tagged and there is only trace of sugar in the capture K-GA or GA stream.

Table 1 Preliminary TEA of K-Ga and Pure GA capture from fermentation broth

Glucaric Extraction from Actual Fermentation Broth

Extraction Stream	Glucarate in Broth (g/kg)	pH of capture stream	Acid Capture Ratio	Energy Consumption (kWh/lb GA)	OPEX ($/ton GA)	CAPEX ($/ton/year GA)	Max. GA Extraction Concentration (wt.%)	Sugar lost in the capture stream
Pure Glucaric Acid	78.3	1.55	50%	1.95	528	1540	52%	< 1%
Glucarate	69.3	7.44	86%	0.67	182	742	58%	< 1%
Glucarate *	73.5	7.54	98%	0.55	147	572	60%	< 1%

* : Extraction using optimal resin wafer material

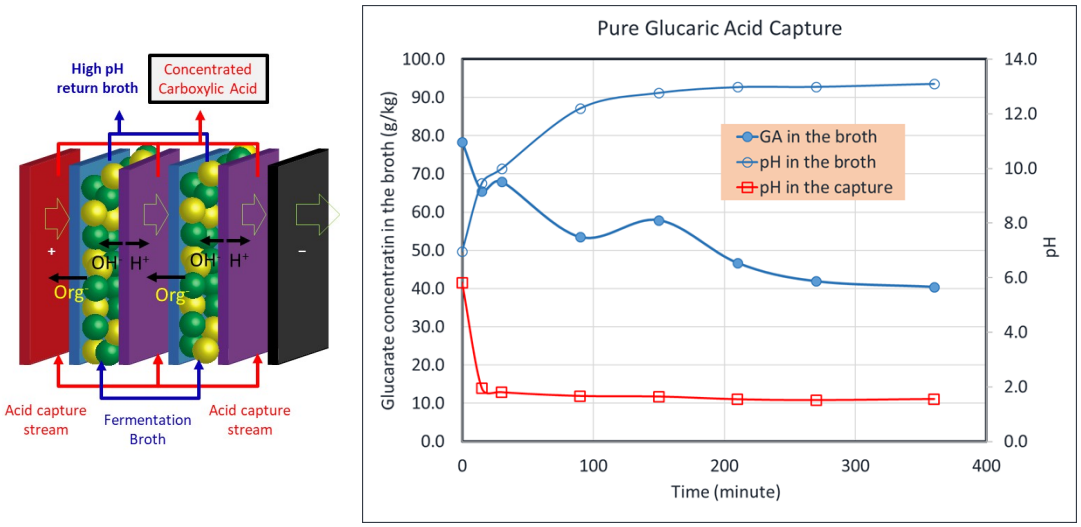

Figure 2 RW-EDI configuration and separation performance of GA capture and purification from Fermentation Broth

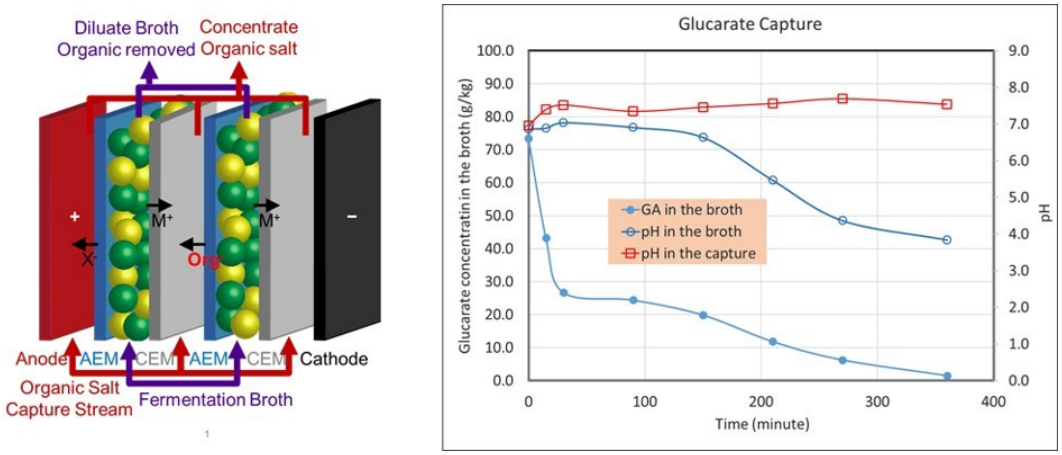

Figure 3 RW-EDI configuration and separation performance of K-GA capture and purification from fermentation broth

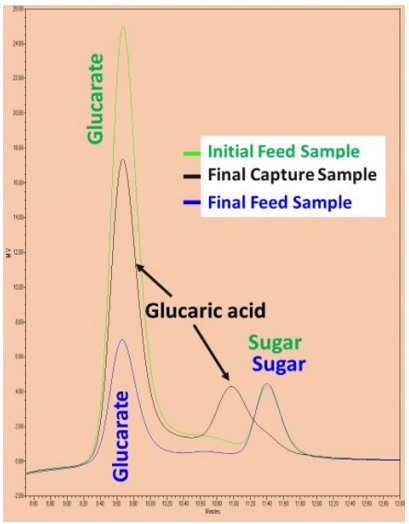

Figure 4 of GA and sugar before and after the separations in the broth and capture stream

ANL (Functionalized Nano-sorbents) – Advanced-materials capture strategies began by developing charts of different nano-sorbent formulations that were likely to be synthetically available to determine (i) which material would bind Kalion products best with carboxylic scavengers, such as TOPO, and (ii) the range of silane monomers available commercially that could facilitate synthesis of materials with tertiary or quaternary amines. Formulations that failed initial synthetic screens were altered by optimization of catalyst molarities and detergent concentrations – alterations that have been successful in other projects. These materials syntheses proved challenging with an overall success rate of 35%, and very few tertiary and quaternary amine materials passed quality control metrics to be used in further evaluation. For the carboxylic-acid scavenging variants, trioctylphosphine oxide (TOPO) was the only scavenger chosen to be studied originally. TOPO is a molecule with three long hydrocarbon tails that will bind with high energy to the surface of the hydrophobic nano-sorbents. As studies progessed, however, two additional scavengers, trioctylamine (TOA) and Cyanex923 were added to the study. Cyanex923 is an interesting proprietary commercial mix consisting of a myriad of different trialklyphosphine oxides. During experiments with

these three scavengers, similar amounts of scavenger could be successfully bound to the surface of the materials (Fig. 5). Unfortunately, materials coated with TOPO were not as efficient in recovering product as materials coated with the other two scavengers (Fig. 6). In comparing the success of the other two scavengers, materials coated with TOA or Cyanex 923 adsorbed very similar amounts of glucaric acid (levels analyzed within desorbed liquid via HPLC; Fig. 7). Unfortunately, materials coated with Cyanex923 tested in low-product-concentration streams co-adsorbed water – severely decreasing product purity in comparison to results with the materials coated with TOA. The co-adsorption of water by materials coated with Cyanex923 was reduced in tests of streams with higher product concentrations but may still prove to be an inferior coating in a production environment. In summary, it appears that materials coated with either TOA or Cyanex923 (or unique combinations thereof) can be used, but Cyanex923 may have some added complications (including the fact that it is a more complex proprietary solution containing multiple trialklyphosphine oxides that potentially could lead to non-specific binding of other chemicals in fermentation broths. The best nano-sorbents may still need a little fine-tuning in order to completely eliminate the need for additional dewatering steps. Alternatively, the nano-sorbent strategy could be combined with RW-EDI or ISPR or membrane approaches to complete the final polishing of the Kalion-process derived products.

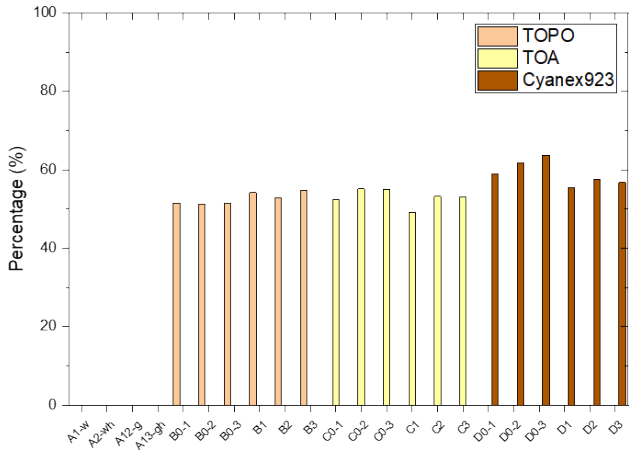

Figure 5 Levels of carboxylic acid scavenger bound to hydrophobic xerogels where coatings could be loaded to comprise 50% or more of the weight of the advanced materials. The A series of readings on the left side of the graph represent controls of various reagents used to facilitate scavenger binding.

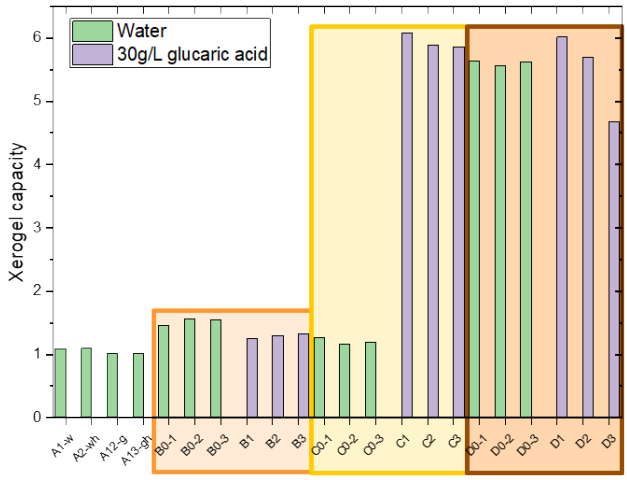

Figure 6 The recovery of glucaric acid (and co-adsorption of water) in mock solutions tested with various hydrophobic nano-sorbents coated with TOPO (orange box), TOA (yellow box) or Cyanex923 (brown box). Recoveries are expressed as the weights of the product recovery relative to the original weight of the dry nano-sorbents. TOA-coated materials proved superior in recovering large amounts of product with reduced co-recovery of water.

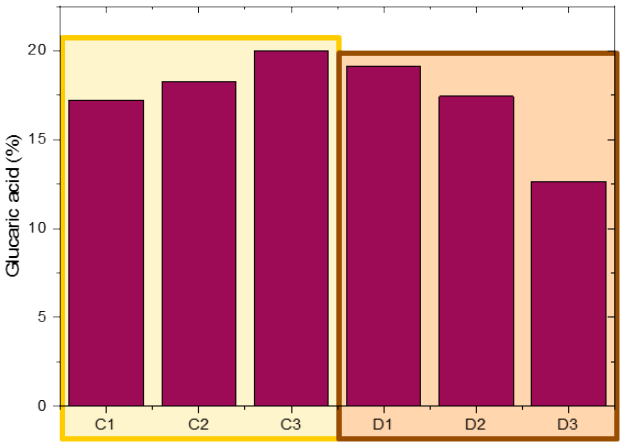

Figure 7 Final concentrations of glucaric acid found in solutions recovered from the surfaces of carboxylic-acid coated nano-sorbents (coloring consistent with labels described in Fig. 6). The materials remained functional through many adsorption/desorption cycles with desorption completed with energies of around 20 psi for ~10s.

ORNL - Membrane pervaporation has been evaluated at different temperatures with model compounds, the highest flux and separation factor were achieved at 70 °C (Table 2). The membrane set up for these experiments is shown in Figure 8. Membrane pervaporation experiments are conducted in a cross-flow mode. The outer-wall polymer coated ceramic tubular membrane is assembled into a concentric stainless-steel tube holder. The membrane-holder assembly contains shell side (feed flow) and membrane tube inner lumen side (permeate side), which is connected with an air vacuum pump

that can supply vacuum in the range of 1–29 in Hg. The temperature-controlled feed (initially 1.25% GA aqueous solution) in a reservoir (500-mL three-neck round bottom glass flask) is pumped out by a peristaltic pump and recirculated flowing through the shell side of the membrane-holder assembly so that the flowing feed solution contacts the outer wall of the membrane tube. At certain time intervals (around 1 hour each) we collect the samples from both permeate side and feed side and the GA concentrations.

For real broth samples, membrane separated Glucaric acid and myoinositol, and water operated for > 10 hours at the optimized condition. The final GA concentration in the feed finally reached to 153 g/L (~15%) by continuously membrane pervaporation.

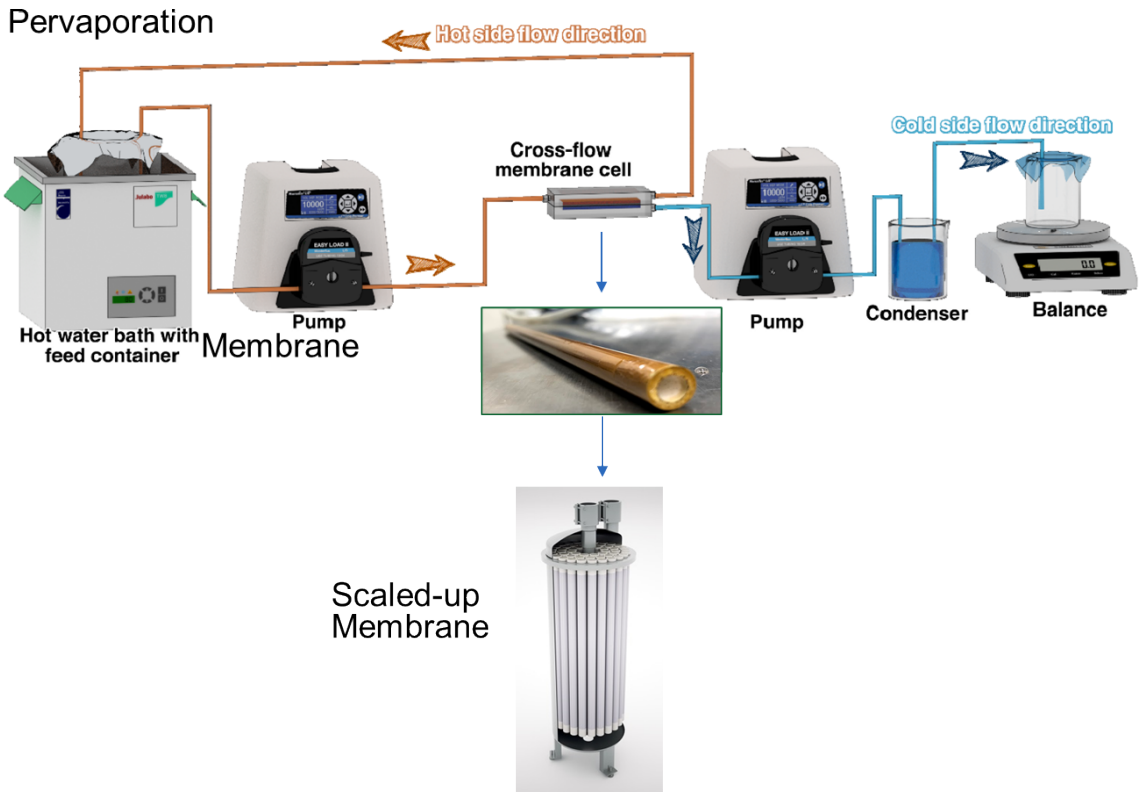

Figure 8. Continuous and scalable membrane separation to concentrate GA/KGA

Table 2 Flux and separation factors for KGA separation at different temperatures via membrane pervaporation

Feed Conc. (KGA in water, wt%)	***Temperature (°C)***	***KGA content in permeate (wt %)***	***Flux (LMH)***	***Separation Factor***
1.25%	50	0.46%	0.36	2.76
	60	0.22%	0.59	5.65
	70	0.18%	0.98	7.00

6. Identify products developed under the award and technology transfer activities, such as:

    a. Publications (list journal name, volume, issue), conference papers, or other public releases of results. If not provided previously, attach or send copies of accepted manuscripts for any public releases to the DOE Project Officer identified in Block 11 of the Notice of Financial Assistance Award;

    NREL *– copy of publication attached.*

    b. Web site or other Internet sites that reflect the results of this project;

    c. Networks or collaborations fostered;

    d. Technologies/Techniques;

    e. Inventions/Patent Applications, licensing agreements; and

    NREL - *Copy of Patent attached*

    f. Other products, such as data or databases, physical collections, audio or video, software or netware, models, educational aid or curricula, instruments or equipment.

7. For projects involving computer modeling, provide the following information with the final report:

N/A

    a. Model description, key assumptions, version, source and intended use;

b. Performance criteria for the model related to the intended use;

c. Test results to demonstrate the model performance criteria were met (e.g., code verification/validation, sensitivity analyses, history matching with lab or field data, as appropriate);

d. Theory behind the model, expressed in non-mathematical terms;

e. Mathematics to be used, including formulas and calculation methods;

f. Whether or not the theory and mathematical algorithms were peer reviewed, and, if so, include a summary of theoretical strengths and weaknesses;

g. Hardware requirements; and

h. Documentation (e.g., users guide, model code).

8. Ensure the report does not contain any Protected Personally Identifiable Information (Protected PII). Protected PII is defined as an individual's first name or first initial and last name in combination with any one or more of types of information, including, but not limited to, social security number, passport number, credit card numbers, clearances, bank numbers, biometrics, date and place of birth, mother's maiden name, criminal, medical and financial records, educational transcripts, etc.

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