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Activity/Case-study 1

Identify alternative microbial-derived protein sources (biomass fermentation) to conventional ones. e.g. mycoproteins/single-cell proteins to vegetal/animal origin proteins.

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This work has been developed under the ERASMUS-EDU-2022-PI-FORWARD Project “*Glocal Ecosystems and Expanded Knowledge for green skills and capability in the Food Sector*” (**G4F**) - Project n° 101087203.
It is credited to the G4F Consortium.

Scenario

- Conventional protein sources, such as animal-based proteins (beef, poultry, and fish) and plant-based proteins (soy, peas, and beans), have long been essential in global nutrition due to their high-quality amino acid profiles and widespread availability. However, their production often leads to significant environmental impacts, including high greenhouse gas emissions, land use, and water consumption, especially in the case of animal proteins. Furthermore, the ethical and sustainability concerns surrounding industrial livestock farming have driven interest in alternative protein sources.
- Mycoproteins and single-cell proteins (SCPs), derived from fungi and yeasts, respectively, present a promising sustainable alternative. These proteins require far less land, water, and energy compared to conventional animal protein sources, and they can be grown on agricultural waste or low-value substrates, reducing food system inefficiencies. However, challenges related to scaling production, nutritional profile and consumer acceptance of these alternative proteins still need to be addressed to fully replace traditional protein sources in the diet.
- Your company, **BioProTech**, has tasked you with developing a sustainable alternative protein source to traditional animal protein sources for a client, a mid-sized food company looking to adopt eco-friendly practices. The company currently sources animal proteins, including beef, poultry, and fish, for its products, which include ready-to-eat meals, burgers, and nutritional supplements. These conventional protein sources come with significant environmental impacts, such as high greenhouse gas emissions, water consumption, and land use.

Task Overview

Working in different teams:

1. **Analyze the suitability of animal-origin proteins and SCPs for high-protein food industry,** considering nutritional profile (e.g. high-quality amino acid profiles) and compatibility with food ingredients, dietary supplements, or high-protein foods (burgers, protein powder supplements).
2. **Evaluate the environmental impact** and **costs** of both protein sources across their lifecycle (e.g., carbon footprint, water and energy consumption).
3. **Propose an alternative-protein source** tailored to the client’s needs, identifying challenges and trade-offs.
4. **Develop a transition plan** for the client, considering economic feasibility, regulatory compliance, and consumer acceptance.

Resources to be provided to the students

- Nutritional profile for animal-origin proteins (beef meat) and SCPs (fungi).
- Summary of LCA data for beef meat and fungi SCPs.
- Case examples of companies that transitioned to fungi-derived SCPs.
- Access to online tools for calculating material carbon footprints.

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For the tutor

Discussion Points Post-Case Study

- What are the limitations of SCPs as a replacement for animal-origin proteins in food industry?
- How can advancements in food biotechnology improve the adoption of sustainable practices?
- What role do consumer perceptions play in the success of alternative protein sources (SCPs) transitions?
- This case study is designed to equip industry professionals with the skills to evaluate and implement alternative-protein sources solutions in the high-protein food products sector, supporting innovation and sustainability.

Thank you

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Resources to be provided to the students

1. NUTRITIONAL PROFILE FOR ANIMAL-ORIGIN PROTEINS (BEEF MEAT) AND SCPS (YEASTS).

Meat is a fundamental component of the human diet, serving as a significant source of high-quality nutrients. It is particularly valued for its rich supply of essential proteins, lipids, vitamins, and minerals, which are critical for numerous physiological processes. The macronutrient profile of meat, characterized by its high biological value proteins and varied fat content, supports tissue synthesis, metabolic regulation, and energy provision. Additionally, its micronutrient composition—specifically B-complex vitamins, iron, zinc, and selenium—plays key roles in enzymatic reactions, oxygen transport, and immune function (Table 1).

According to European legislation, meat refers to the edible parts of livestock species such as cattle, pigs, sheep, and poultry. Various factors, including animal species, breed, diet, environmental conditions, and the anatomical origin of the cut influence the nutritional attributes of meat. For instance, lean meat cuts, such as chicken breast or turkey, provide higher protein concentrations and lower lipid content, whereas fatty cuts yield greater energy density due to elevated fat levels (R. S. Ahmad, Imran, Hussain, & nutrition, 2018).

Meat Cut	Protein (g)	Sat. Fat (g)	Fat (g)	Energy (kcal)	Vit. B12 (mcg)	Na (mg)	Zn (mg)	P (mg)	Fe (mg)
Chicken breast, raw	24.2	0.2	8.5	178	0.39	71	0.9	199	1.2
Beef, steak cuts, raw	21.0	1.9	4.5	123	1.9	59	1.7	167	1.3
Chicken, raw	22.8	0.6	1.9	113	0.70	78	1.4	202	0.7
Pork, chop, raw	18.1	10.8	31.7	353	1.1	60	1.8	190	1.4
Turkey, skinless, raw	19.9	1.8	7.1	136	1.9	42	1.5	209	2.1

The amino acid profile of beef meat represents a comprehensive and bioavailable source of essential and non-essential amino acids, which are fundamental for various physiological functions, including protein synthesis, enzymatic activities, and tissue repair. ***Beef is particularly valued for its high content of branched-chain amino acids*** (such as leucine, isoleucine, and valine) that are crucial for muscle metabolism, as well as lysine and methionine, which are vital for collagen formation and metabolic processes. The precise composition and concentration of these amino acids can vary among different beef cuts, reflecting differences in muscle composition and biochemical properties, thereby influencing the meat's nutritional and functional qualities. The table below presents the amino acid composition of different beef cuts (Chuck, Round, and Loin), highlighting variations in their nutritional profiles based on dry weight measurements (Wu et al., 2016).

Table 1. Amino acid profile of beef meat

Amino Acid	Chuck (mg/g dry weight)	Round (mg/g dry weight)	Loin (mg/g dry weight)
Essential Amino Acids (EAAs)			
Histidine	29.4	31.0	31.7
Isoleucine	38.4	40.5	41.1
Leucine	61.8	65.1	66.7
Lysine	66.6	70.4	72.0
Methionine	23.7	24.8	25.3
Phenylalanine	30.9	33.1	33.5
Threonine	34.3	35.8	37.0
Tryptophan	9.34	9.77	10.0
Valine	44.8	46.9	47.4
Non-Essential Amino Acids (NEAAs)			
Alanine	42.2	44.5	45.4
Arginine	47.9	51.0	52.4
Asparagine	30.3	32.9	33.4
Aspartate	37.7	40.3	41.1
Cysteine	10.1	10.8	11.2
Glutamate	68.9	73.8	75.1
Glutamine	46.8	48.5	49.9
Glycine	31.0	33.3	33.7
Proline	30.0	31.5	32.9
Serine	32.0	34.2	35.4
Tyrosine	27.1	28.9	30.1
4-Hydroxyproline	1.73	1.74	1.77

The term ***"single cell protein" (SCP)*** is widely regarded as the most accurate descriptor, as it refers to protein produced by single-celled organisms. Yeast, for example, has been shown to be capable of ***producing approximately 250 tons of protein*** within 24 hours. The concept of SCP was first introduced in 1968 during a meeting at the Massachusetts Institute of Technology (MIT), where researchers sought to identify a more precise term to replace previously used terminology, such as "microbial protein". Various microbial species, including algae, bacteria, fungi, and yeast can synthesize SCP. In addition to its high protein content, which ranges from 60% to 82% on a dry matter basis, single cell protein (SCP) also contains carbohydrates, nucleic acids, lipids, minerals, and vitamins (Sharif et al., 2021). Yeast for example is a good choice for SCP production as it grows rapidly on different sugars-rich feedstocks. Moreover, most yeasts are safe for consumption. The most common mycoprotein product on the market, Quorn^{TM}, has been produced since 1985 following 15 years of research and development (M. I. Ahmad, Farooq, Alhamoud, Li, & Zhang, 2022). ***Fusarium venenatum***, a fungus, has been cultivated in England for more than a decade to produce mycoprotein, commercially known as "Quorn." This product boasts a fibrous texture and is an excellent source of high-quality protein, including all essential amino acids***. Fusarium venenatum***-derived mycoprotein comprises approximately 44% protein by weight on a dry basis, and its net protein utilization (NPU) is similar to that of milk.

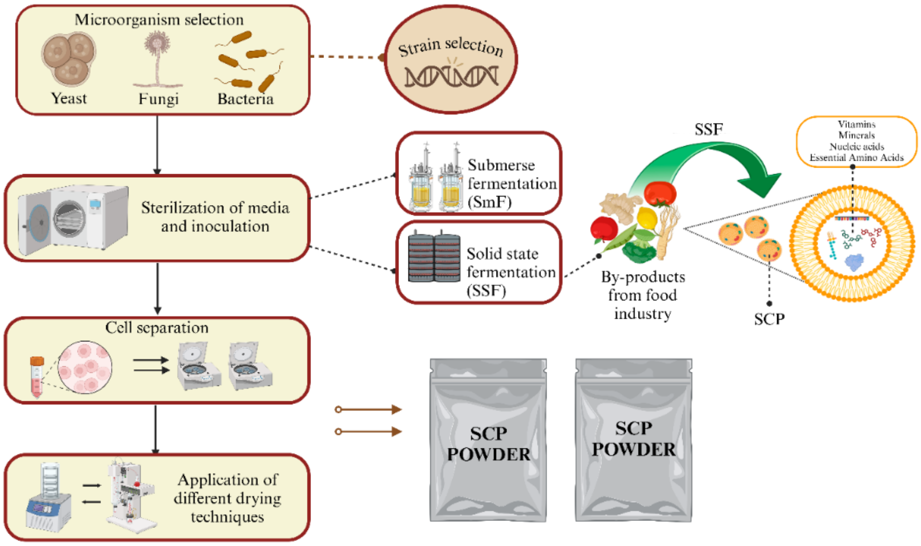

Fig 1. Schematic representation of the process for the production SCP (Source: Original)

The quantity and quality of single-cell protein (SCP) are influenced by the microorganism used and the culture conditions. For instance, ***the protein yield from microalgae can vary significantly, ranging from 30% to 80%***. SCP generally contains a higher percentage of protein compared to conventional food sources, such as soy (38.6%), fish (17.8%), meat (21.2%), and whole milk (3.28%). Table 2 presents a comparison of protein and amino acid content between conventional foods and SCP. SCP derived from *Haematococcus pluvialis* and *Saccharomyces cerevisiae* is a rich source of threonine (up to 7.41%) and tryptophan (up to 14.22%), both of which are considered limiting amino acids in milk and meat, respectively. ***SCP also provides essential amino acids such as methionine, threonine, and lysine, with lysine being deficient in cereals,*** suggesting that combining SCP with cereals could enhance the nutritional value of the food. Studies have shown that SCP protein can surpass the protein content of other sources, potentially offering health benefits to organisms that consume it. For example, supplementing the diet of *Jian carp* (Cyprinus carpio var. Jian) with the methanotroph *Methylococcus capsulatus* (Bath) significantly improved their mean final weight, rate of weight gain, specific growth rate, and serum antioxidant capacity while reducing malondialdehyde production, compared to fish fed soybean meal (Salazar-López et al., 2022).

Protein composition (%)	Meat (Beef)	Milk (Cow)	Fish (Carp, raw)	*Rhodopseudomonas faecalis* (Bacterium)	*Candida utilis* (Yeast) and *Brevibacterium lactofermentum* (Corynebacterium)	*Haematococcus pluvialis* (Microalgae)
TOTAL PROTEIN (%)	21.2	3.28	17.8	51.5	54.5	64.93
Essential amino acids (%)						
Isoleucine	2.41	0.12	2.71	3.7	3.45	2.58
Leucine	4.06	0.23	4.35	7.6	4.13	10.87
Lysine	4.45	0.13	5.16	5.6	25.00	11.05
Methionine	1.35	0.04	1.62	0.5	1.86	0.54
Phenylalanine	2.20	0.13	2.22	4.1	1.65	3.17
Threonine	2.29	0.08	2.59	0.3	3.93	7.41
Tryptophan	–	–	–	3.8	–	–
Valine	2.50	0.14	3.46	5.5	3.84	–
Histidine	1.70	0.06	2.00	1.9	0.79	1.84
Non-essential amino acids (%)						
Cysteine	0.64	–	0.66	1.0	–	1.19
Tyrosine	1.80	0.12	2.07	2.5	2.49	6.91
Arginine	3.16	0.05	3.21	1.1	3.35	21.44
Alanine	2.92	0.08	3.39	6.6	2.82	12.68
Aspartic acid	4.50	0.13	5.86	4.7	4.87	18.71
Glutamic acid	7.65	0.35	7.99	3.7	12.00	5.62
Glycine	2.43	0.04	2.73	6.1	3.87	28.12
Proline	1.89	0.15	2.08	5.4	2.74	9.96
Serine	2.02	0.10	2.45	3.7	1.24	7.70
Asparagine	–	–	–	–	–	6.67
Glutamine	–	–	–	4.3	–	6.83

2. SUMMARY OF LCA DATA FOR BEEF MEAT AND YEAST SCPS.

A nationwide Life Cycle Assessment (LCA) was initiated as part of the US Beef Sustainability Research Program to establish baseline environmental impact metrics and identify opportunities for improvement within the beef value chain. Primary cradle-to-farm gate inventory data were collected from the Roman L. Hruska US Meat Animal Research Center (USMARC), the largest agricultural animal research facility in the United States. The primary objective of this LCA was to establish a baseline for the environmental impacts associated with current practices throughout the US beef value chain. Specifically, the study aimed to quantify the sustainability impacts linked to the production and consumption of 1 kg of beef in a representative US system. The target audience for the results included stakeholders within the beef industry, consumers, and the broader public.

Table 2. Emission rates from fertilizer and manure application on feed crops used in US beef life cycle impact assessment

Emission Type	Rate
Runoff loss (corn fields only)	0.15 g P/kg P applied 0.60 g N/kg N applied
Air emissions (direct + corn crop residue)	0.20 g N2O/kg applied
N fertilizer leaching	30% of N applied
Leached N to N_2O-N	0.75% (2.25 kg N_2O-N/kg fertilizer N applied)
CO_2 from urea	200 g CO2-C/kg $(NH_2)_2CO$ applied
CO_2 from limestone	120 g CO_2-C/kg $CaCO_3$ applied
Volatilization of NH_3 from fertilizer-N	100 g NH_3/kg N applied

N2O-N = annual direct N2O-N emissions produced from soil amendment (urea or limestone) decomposition, kg N2O-N/year

CO2-C emission = annual C emissions from soil amendment (urea or limestone) decomposition, kg C/year

The Life Cycle Assessment (LCA) results (Table 3) for the beef value chain revealed that the feed and cattle production phases were the predominant contributors to most environmental impact categories. Key impact metrics included ***water emissions*** (7005 L diluted water equivalent per kilogram of beef [L eq/CB]), ***cumulative energy demand*** (1110 MJ per kilogram of beef [MJ/CB]), and ***land use*** (47.4 m^2 per kilogram of beef per year [m^2 a eq/CB]). Air emissions were quantified in terms of ***acidification potential*** (726 g SO_2 equivalent per kilogram of beef [g SO_2 eq/CB]), ***photochemical ozone creation potential*** (146.5 g C_2H_4 equivalent per kilogram of beef [g C_2H_4 eq/CB]), ***global warming potential*** (48.4 kg CO_2 equivalent per kilogram of beef [kg CO_2 eq/CB]), and ***ozone depletion potential*** (1686 µg CFC-11 equivalent per kilogram of beef [µg CFC-11 eq/CB]). Other metrics included ***abiotic depletion potential*** (10.3 mg Ag equivalent per kilogram of beef [mg Ag eq/CB]), ***consumptive water use*** (2558 L equivalent per kilogram of beef [L eq/CB]), ***and solid***

waste (369 g municipal waste equivalent per kilogram of beef [g municipal waste eq/CB]). ***In terms of relative contribution, the feed phase accounted for 0.93 of the human toxicity potential***.

Table 3 Environmental impact metrics quantified in the life cycle assessment of US beef where 1 unit of consumer benefit (CB) is equivalent to 1 kg of consumed, boneless, edible beef in the USA

Impact	Units	Feed	Cow-calf	Finish	Packing	Case ready	Retail	Consumer	Restaurant
Abiotic depletion potential	mg Ag eq/CB	1.51	3.95	2.68	0.24	0.16	0.14	0.59	1.01
Cumulative energy demand	MJ/CB	988.0	11.6	6.0	1.4	8.3	6.6	29.3	48.4
Consumptive water use	L eq/CB	2506	11.9	11.2	3.7	1.9	1.7	6.8	14.0
Absolute consumptive water use	L abs./CB	5023	23.9	22.5	7.5	3.9	3.4	13.7	28.1
Human toxicity potential	norm. tox. pts.	0.93	0.034	0.027	0.003	0.002	0.001	0.001	0.002
Land use	m^2 a eq/CB	45.8	0.8	0.7	0.1	0.2	0.0	0.1	0.2
Acidification potential	g SO_2 eq/CB	127.4	359.2	210.7	2.6	1.7	2.3	7.8	13.9
Global warming potential	kg CO_2 eq/CB	7.42	28.51	6.39	0.55	0.27	0.46	2.01	2.83
Ozone depletion potential	μg CFC_{11} eq/CB	121.4	0.1	1.4	36.9	336.6	180.7	9.0	1008
Photochemical ozone creation potential	g C_2H_4 eq/CB	136.9	6.8	1.8	0.2	0.1	0.2	0.4	0.1
Solid wastes	g municipal waste eq/CB	91.3	101.4	21.5	45.1	7.0	10.1	25.3	67.3
Water emissions	L diluted water eq/CB	6127	17.9	2.4	126.1	484.9	2.2	198.8	45.9
Abiotic depletion potential	mg Ag eq/CB	1.51	3.95	2.68	0.24	0.16	0.14	0.59	1.01

This LCA is the first comprehensive assessment of its kind for beef production and has been third-party verified in accordance with ISO 14040:2006, ISO 14044:2006, and ISO 14045:2012 standards. A subsequent nationwide study of beef cattle production is currently underway, utilizing region-specific data to establish benchmarks at the regional level and identify opportunities for further improvements in the sustainability of US beef production (Asem-Hiablie, Battagliese, Stackhouse-Lawson, & Alan Rotz, 2019).

Yeast-based single-cell protein (SCP) produced using oat side-streams as feedstock (OS-SCP) demonstrates notable differences in environmental impact when compared to other protein sources. ***OS-SCP results in a 61% reduction in land*** use compared to conventional products such as soy protein concentrates. However, it exhibits higher environmental impacts across several categories, including ***global warming potential (205–754% increase)***, ***water consumption (166–1401% increase)***, ***freshwater eutrophication (118–333% increase)***, and ***terrestrial acidification (85–340% increase)***. When compared to other novel protein sources, such as yeast protein concentrate, methanotrophic bacterial SCP, and insect meal, OS-SCP shows a more significant environmental footprint. Nevertheless, OS-SCP also demonstrates a reduction in global warming (11% decrease) and freshwater eutrophication (20% decrease) compared to dry microalgae biomass(Kobayashi et al., 2023).

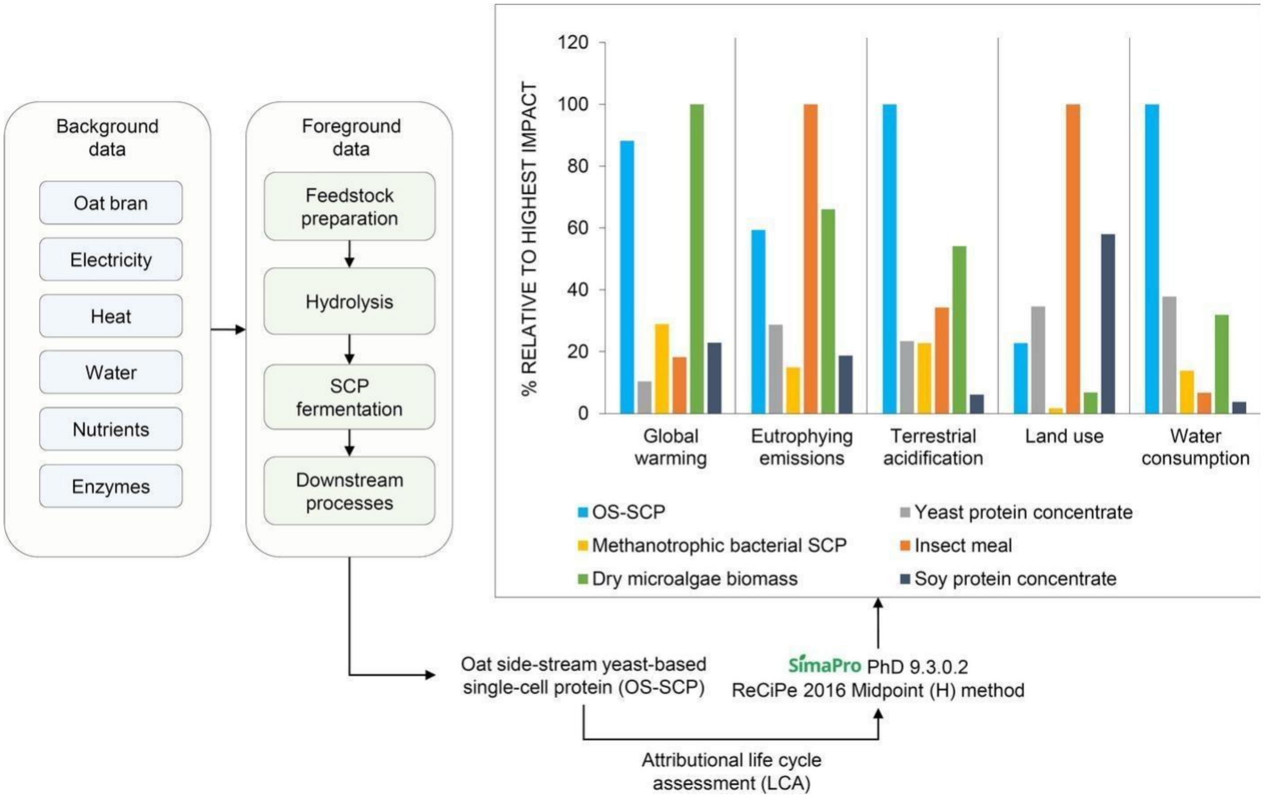

Figure 2. Comparative Environmental Footprint of OS-SCP vs. Other Novel Protein Sources

The production process for single-cell protein (SCP) derived from biomethane emerged as the dominant contributor in five out of six evaluated environmental impact categories: ecotoxicity (42.5%), global warming potential (39.2%), human toxicity—cancer (59.5%), human toxicity—non-cancer (48.3%), and water scarcity (70.1%). In contrast, the landfilling of rejected materials from the pretreatment stage was identified as the primary driver of freshwater eutrophication. Given the SCP production process's significant contribution to these impact categories, a detailed assessment of its operations is imperative. Analysis revealed that electricity consumption is the critical factor responsible for its environmental burden, contributing between 43.7% and 72.7% across five of the six impact categories, including ecotoxicity, eutrophication, global warming, and both cancer and non-cancer human toxicity.

To mitigate these environmental impacts, transitioning to renewable energy sources is strongly recommended. For instance, a study by Järviö et al. reported an 88% reduction in global warming potential for microbial protein production using autotrophic hydrogen-oxidizing bacteria (HOB) when hydropower replaced the conventional electricity mix in Finland. This highlights the transformative potential of integrating renewable energy into SCP production to reduce its environmental footprint (Fernández Gutiérrez, Argüelles, Martínez, Disla, & Lara-Guillén, 2022).

Table 4. Life Cycle Inventory for the single-cell protein to obtain 1 kg of protein from microorganisms (SCP)

Stage	Inputs	Outputs	Emissions to Air
Biowaste Pretreatment and Anaerobic Digestion	Biowaste: 71.4 kg FeCl3: 0.1 kg Polyelectrolyte: 0.04 kg Tap water: 28.8 kg Diesel: 0.09 kg Electricity: 12.0 MJ	Leftover biogas (to upgrade): 7.1 kg Solid fraction of digestate: 47.5 kg Liquid fraction of digestate: 39.2 kg Rejected materials to landfill: 6.4 kg	CH_4: 0.083 kg CO: 1.07 g CO_2: 0.296 kg NO_x: 1.96 g N_2O: 0.014 g SO_2: 6.00×10^{-3} g NH_3: 8.00×10^{-4} g NMVOCs: 0.156 g PM_{10}: 0.079 g $PM_{2.5}$: 0.079 g
SCP Production	Chemicals: 62.38 kg	Protein from microorganism: 1.00 kg Uniprotein (8% H2O) : 1.55 kg Wastewater to wastewater treatment plants: 8.6 kg CH_4: 0.057 g	

		CO: 2.23 g CO_2: 1.019 kg NO_x: 5.08 g N_2O: 6.00×10^{-3} g NMVOCs: 0.15 g PM10: 0.051 g PM2.5: 0.051 g	

The term “Chemicals” includes biomethane, oxygen, water, natural gas, sodium hydroxide, and the sources of nitrogen, phosphorus and sulfur.

3. CASE EXAMPLES OF COMPANIES THAT TRANSITIONED TO YEAST-DERIVED SCPS.

Quorn

- Background: Quorn is a global leader in meat alternatives, producing mycoprotein-based products derived from fungal SCP.
- Transition Details: The company pioneered the use of fermentation to produce mycoprotein, which is cultivated from Fusarium venenatum, a type of fungus, and blended into various meat substitute products.
- Impact: Quorn's SCP-based products offer a sustainable protein source that uses significantly less water, land, and energy compared to traditional meat production, helping address climate change and resource scarcity.
- Web site: https://www.quorn.co.uk/mycoprotein

Quorn mycoprotein is a sustainable, meat-free protein source that is rich in dietary fiber and low in saturated fat. Its production begins not with livestock but with Fusarium venenatum, a natural, nutrient-dense filamentous fungus found in soil. The manufacturing process utilizes fermentation, a traditional biotechnological technique commonly employed in the production of bread, beer, and yogurt. Through controlled fermentation, Fusarium venenatum is cultivated to produce mycoprotein efficiently. The environmental advantages of ***Quorn mycoprotein are significant; its production generates 95% less*** CO_2 ***compared to conventional beef mince***, making it a highly sustainable protein option. This approach represents an innovative and eco-friendly solution to meet the nutritional needs of a growing global population while reducing the environmental impact of protein production.

Unibio Group

- Background: Unibio Group is a biotechnology company that focuses on producing sustainable protein using microbial fermentation, including yeast-based SCP.
- Transition Details: The company developed its proprietary U-Loop® technology, which converts methane into SCP for use as animal feed. Their product, Uniprotein®, offers a high-protein, sustainable alternative to traditional feeds like soy and fishmeal.

- Impact: By utilizing methane as a feedstock, Unibio significantly reduces land and water use, offering a lower-carbon solution to meet the growing global protein demand.
- Website: https://www.unibio.dk/end-product/protein/

Uniprotein® is a high-quality, protein-rich biomass containing approximately 72% protein, designed as a direct supplement for animal feed formulations. It is presented as a free-flowing, reddish-brown granule with a particle size range of 150–200 μm and features an extended shelf life. The production process ensures consistent product uniformity. Derived from a natural, non-genetically modified process industrialized by Unibio, Uniprotein® is produced through microbial fermentation using natural gas as the sole carbon and energy source. This method is environmentally sustainable, as the only by-product is clean water. Uniprotein® is free from toxins, dioxins, and heavy metals, owing to its highly controlled production environment and the exclusive use of food-grade minerals. Its innovative production process highlights its potential as a sustainable and safe alternative protein source for the animal feed industry.

4. ACCESS TO ONLINE TOOLS FOR CALCULATING MATERIAL CARBON FOOTPRINTS.

SIMAP (Sustainability Indicator Management & Analysis Platform)

Description: A comprehensive tool for assessing carbon footprints in supply chains, energy use, and material inputs. Institutions and companies often use it.

Website: https://unhsimap.org/

PlanBe.Eco Carbon Footprint Calculator

Description: PlanBe.Eco offers a user-friendly carbon footprint calculator designed to assess and mitigate environmental impacts. This platform caters to both individuals and businesses, emphasizing simplicity and actionable insights for sustainability goals.

Website: PlanBe

Carbon Trust Footprint Calculator

Description: Offers a user-friendly interface to calculate the carbon footprint of materials and products. It is designed for businesses and organizations seeking to estimate their emissions.

Website: https://www.carbontrust.com/en-eu

OpenLCA

Description: A free and open-source software for life cycle assessment (LCA). Users can model carbon footprints of materials and products using publicly available databases.

Website: https://www.openlca.org/

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Activity/Case-study 2

Proposing an Innovative Solution for Controlled Fermentation-Derived Products

A Holistic Perspective on Applications, Environmental Impact, Raw Materials, and Cost

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Scenario

- Citric acid, widely used in the food industry as a **flavor enhancer, preservative, and acidity regulator**, is predominantly produced via **microbial fermentation**. This process utilizes **sugar-rich feedstocks**, such as corn, molasses, or cassava starch, as the primary substrate. While this method is efficient and cost-effective, it is heavily reliant on agricultural inputs, which can contribute to land use, resource depletion, and food security challenges.
- Your client, a mid-sized food manufacturer "GreenCycle Ingredients" specializing in ready-to-eat meals, snacks, and beverages, is looking to make their production processes more sustainable. They currently rely on buying the commercially available citric acid but seek a cleaner and more circular approach to produce it themselves. Their goal is to leverage **food waste and by-products** generated during their manufacturing processes (e.g., fruit peels, pulp, vegetable scraps from beverages production) as alternative substrates for citric acid fermentation. This aligns with their commitment to **reduce waste, achieve net-zero emissions, and transition to a circular**
- **Key client details:**
- Their current supply of citric acid depends on external suppliers using traditional fermentation methods, adding significant transportation-related emissions and costs.
- They generate substantial waste during their operations, including citrus peels, starchy residues, and other organic by-products, which are currently discarded or underutilized.
- The company is committed to achieving **sustainability goals** by 2030, including waste reduction and a focus on natural ingredients.

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Task Overview

Working in different teams:

1. Analyze the suitability of circular fermentation for citric acid production

Evaluate food waste as a substrate for fermentation. Assess citric acid's functionality (flavor, acidity, preservation) and compare waste-derived citric acid with conventional production in performance and quality.

2. Evaluate the environmental impact and costs

Analyze carbon footprint, water, and energy savings of waste-based citric acid versus conventional methods. Assess economic feasibility, cost savings, and scale-up challenges of using food waste as feedstock.

3. Propose a fermentation solution

Design a scalable process to convert food waste into citric acid, addressing feedstock variability, quality consistency, and regulatory compliance. Propose innovations to boost yield and cut costs.

4. Develop a transition plan for circular citric acid production

Develop a roadmap for fermentation-based citric acid, covering food safety certification, investment strategies, economic feasibility, and consumer engagement to promote sustainability benefits.

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Resources to be provided to the students

- **Chemical and functional profiles** - properties of citric acid and its applications in food products (e.g., preservation, flavor, and acidity regulation)
- **Raw material data –** composition and availability of food waste feedstocks
- **LCA** data for citric acid production
- **Case studies of circular fermentation for citric acid** - examples of successful food companies adopting waste-to-value solutions.
- **Access to analytical tools**
- **Regulatory guidelines**

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Deliverables to be provided by the students

Each team will produce:

- A detailed report on their assigned aspect.
- A visual presentation summarizing key findings and proposed solutions.
- A collaborative proposal outlining a comprehensive fermentation-based solution.

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Teacher’s Role

- **Facilitator**: Guide students through the problem-solving process by posing questions and offering clarifications.
- **Evaluator**: Assess the feasibility, creativity, and thoroughness of proposed solutions.
- **Discussion Leader**: Encourage critical reflection on challenges, trade-offs, and potential advancements in fermentation technology.

Holistic perspective

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A. Applications

Preservative for beverages, sauces, and processed foods.

Flavor enhancer in ready-to-eat meals, snacks, and confectionery.

Acidity regulator in baked goods, jams, and dairy products.

Suitable for industries seeking natural replacements for synthetic additives.

B. Environmental perspective

Reduces food waste by using by-products like citrus peels and pulp as feedstock.

Supports circular economy goals by minimizing reliance on virgin agricultural inputs.

Lowers greenhouse gas emissions compared to synthetic citric acid production.

Helps divert waste from landfills, reducing methane emissions.

C. Raw materials

Food Industry By-Products: Citrus peels, fruit pulp, and starchy residues.

Non-Food Industry Contributions: Organic waste streams suitable for fermentation.

Pre-Treatment Requirements: Enzymatic hydrolysis to convert waste into fermentable sugars.

D. Regulatory Requirements

Compliance with food safety standards (e.g., EFSA, FDA) for waste-derived ingredients.

Alignment with clean-label certification to meet consumer demand for natural products.

Upcoming regulations (e.g., EU Waste Framework Directive) supporting waste-to-value transitions.

E. Cost considerations

Cost comparison between traditional citric acid production (from sugar-rich crops) and waste-based fermentation.

Assessment of scalability for small- and large-scale manufacturers.

Evaluation of cost-efficiency improvements from integrated waste-to-value processes (e.g., reduced raw material costs).

F. Consumer perspective

Willingness to pay: Higher acceptance for natural, sustainable citric acid among eco-conscious consumers.

Safety assurance: Clear communication about the safety of waste-derived citric acid.

Sustainability: Market appeal driven by its role in reducing food waste and carbon footprint.

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Interactive discussion/Stakeholder perspectives

Food manufacturers

- Does the innovation meet clean-label and sustainability demands from retailers and consumers?
- Can manufacturers market products as natural and eco-friendly while maintaining competitive pricing?

Environmentalists

- Does the shift toward waste utilization strengthen broader sustainability efforts?
- How does this solution influence consumer awareness about waste reduction and environmental impacts?

Regulators

- Are policies in place to support consumer-driven trends toward natural and sustainable ingredients?
- Can the innovation drive broader changes in waste management and resource efficiency?

Consumers

- Are consumers willing to pay a premium for waste-derived, eco-friendly products?
- How does this align with the growing demand for transparency and sustainability in food production?

Resources to be provided to the students

Functional profile of citric acid

- Key properties and applications of citric acid in the food industry (e.g., flavor enhancement, acidity regulation, preservation).

Summary of LCA data

- Comparative environmental impact data for citric acid produced via traditional methods (chemical synthesis) versus fermentation using food waste.

Examples of industry transitions

- Case studies of companies successfully implementing fermentation-based citric acid production, including their challenges and solutions.

Raw material profiles

- Data on potential feedstocks for citric acid fermentation (e.g., citrus peels, fruit pulp, starchy residues) and their conversion efficiency.

Economic analysis tools

- Access to templates or tools for evaluating the cost-effectiveness and scalability of fermentation-based citric acid production.

Regulatory guidelines and standards

- Information on food safety certifications and clean-label compliance for citric acid derived from fermentation.

Environmental and circular economy metrics

- Tools to assess resource efficiency, waste reduction, and carbon footprint savings from fermentation-based citric acid production.

Market insights and consumer preferences

- Research on consumer trends for sustainable and natural ingredients, with a focus on citric acid.

Innovation frameworks

- Methodologies for designing innovative fermentation solutions tailored to citric acid production, including waste-to-value strategies.

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For the tutor

Discussion Points Post-Case Study

- What are the main challenges of using food waste as a substrate for citric acid fermentation?
- How can advancements in fermentation technology improve the scalability and efficiency of waste-based citric acid production?
- What strategies can help address regulatory and consumer acceptance challenges for waste-derived food ingredients?
- What are the economic and environmental trade-offs of transitioning to a circular fermentation model?
- How can companies collaborate across the value chain to maximize the benefits of circular fermentation?

Key Features of the Solution

1. Raw materials: utilization of food and agricultural waste feedstock:

- Use of food production by-products such as citrus peels, fruit pulp, and starchy residues as fermentation substrates.
- Pre-treatment technology: deployment of green technologies to convert waste into fermentable sugars, ensuring optimal substrate quality.
- Environmental impact: reduces reliance on virgin agricultural inputs, diverts food waste from landfills, and minimizes methane emissions.

2. Process design: IoT-enabled automation

- Smart bioreactors: equipped with real-time sensors to monitor pH, temperature, nutrient levels, and product concentration during citric acid fermentation.
- Automated control systems: machine learning algorithms adjust fermentation parameters to optimize citric acid production efficiency.
- Scalability: modular designs support seamless scaling from pilot to industrial production for citric acid.

3. Circular economy: integrated downstream processing

- Energy recovery: capture of off-gases like CO_2 for reuse in beverage carbonation or other applications.
- Water recycling: advanced filtration systems recycle water used in the fermentation process to minimize waste.
- Waste minimization: residual biomass from fermentation repurposed as bioenergy or agricultural fertilizer, completing the waste-to-value cycle.

Thank you

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Resources to be provided to the students

1. CHEMICAL AND FUNCTIONAL PROFILES

Citric acid (CA), the primary organic acid produced through fermentation today, has its origins traced back to 1784 when W. Scheele first isolated it from lemon juice. He achieved this by precipitating calcium citrate, which, upon treatment with sulfuric acid, yielded citric acid in the liquid phase. Citric acid is a tricarboxylic acid, specifically ***propane-1,2,3-tricarboxylic acid*** (figure 1), with a hydroxyl group attached at position 2. It serves as a vital metabolite in the metabolic pathways of all aerobic organisms. Citric acid functions as a ***food acidity regulator, chelating agent, antimicrobial agent, and fundamental metabolite***. It also acts as the conjugate acid of both citrate(1-) and citrate anion (PubChem). Anhydrous citric acid is highly soluble in water, freely soluble in ethanol, and sparingly soluble in ether, while monohydrate CA is soluble in water and sparingly soluble in ether. At room temperature, it exists as a solid, melts at 153°C, and has a boiling point of 310°C (Kubicek, 1998). Upon heating beyond approximately 175°C, it decomposes with the release of carbon dioxide (CO_2). When dissolved in water, citric acid exhibits weak acidity but imparts a strong acidic taste, enhancing sweetness and contributing a fruity tartness. This characteristic is widely utilized to complement fruit flavors in the food and beverage industry. In conjunction with citrate, citric acid provides excellent buffering capacity, while its remarkable chelating properties for metal ions enhance its physicochemical versatility. These attributes make it an indispensable component in various applications, including food, cosmetics, nutraceuticals, and pharmaceuticals, highlighting its multifaceted utility (Behera, Mishra, & Mohapatra, 2021)

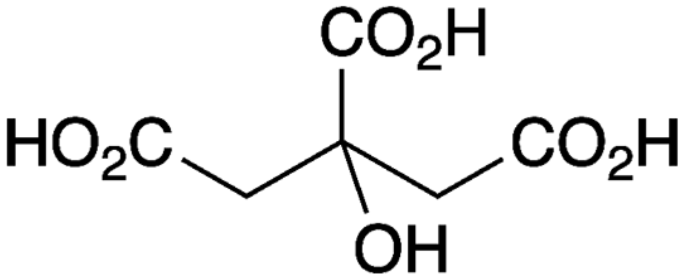


Figure 1. Citric acid chemical structure

Approximately 60% ***of citric acid production is utilized in the food and beverage industry*** due to its designation as ***Generally Recognized as Safe (GRAS)***, appealing taste, high water solubility, and chelating and buffering capabilities. In carbonated beverages, citric acid is extensively employed to enhance flavour profiles and complement fruit and berry flavours while increasing the efficacy of antimicrobial preservatives. The concentration of citric acid used varies depending on the product's flavour profile, typically ranging from 1.5% to 5%(Berovic & Legisa, 2007).

In jams and jellies, citric acid is essential for flavor enhancement and precise pH adjustment, which is critical for optimal gelation. The pH must be maintained within narrow limits to ensure proper consistency, and citric acid is often introduced as a 50% solution to ensure uniform distribution throughout the batch (Fukui & Tanaka, 1980).

In the confectionery sector, citric acid is a flowing agent in concentrations of 0.5% to 2.0% . Its chelating and pH-adjusting properties improve the stability of frozen food products by enhancing antioxidant activity and deactivating enzymes, which help preserve product quality and extend the shelf life of frozen fish and shellfish. Additionally, citric acid prevents the degradation of colour and flavour ***in frozen fruits***.

In fats, oils, and fat-containing foods, it acts as an antioxidant synergist when used in concentrations of 0.005% to 0.02%. Furthermore, citric acid is a flavour enhancer in sherbets and ice creams, contributing to their sensory appeal (Berovic & Legisa, 2007).

Citric acid is widely used in ***cosmetic formulations*** as a standard ingredient for pH adjustment and as a chelating agent in antioxidant systems, effectively binding metallic ions. Its detergent-building properties make citrate an eco-friendly, biodegradable alternative to phosphates in non-phosphate detergent powders, addressing environmental concerns.

In metal cleaning applications, citric acid-based formulations efficiently remove oxidation products from the surfaces of ferrous and non-ferrous metals, ensuring effective surface preparation. Additionally, citrates play a significant role in metal plating processes, aiding in the deposition of metals such as copper, nickel, chromium, lead, and other heavy metals.

The majority of citric acid production is achieved through biological processes, primarily *via* submerged fermentation of starch- or sucrose-based media, such as molasses, using the filamentous fungus ***Aspergillus niger***. This organism is favoured due to its high citric acid productivity at low pH levels without generating toxic by-products. ***Citric acid is classified as a metabolite of energy metabolism (Figure 2), with its accumulation occurring only under conditions of significant metabolic imbalances.*** In recent years, attention has shifted toward the utilization of agricultural waste residues and by-products as alternative substrates for citric acid production through solid-state fermentation (SSF), offering a sustainable and cost-effective approach.

The TCA cycle (Figure 2), also known as the citric acid cycle, is a fundamental metabolic pathway found in the mitochondria of aerobic organisms. It is catalyzed by a multi-enzyme system that processes acetyl-CoA, breaking it down to release carbon dioxide and hydrogen atoms. ***During each cycle, one molecule of acetyl-CoA (containing two carbon atoms) combines with the four-carbon compound oxaloacetate to form citric acid, a six-carbon compound.*** This citric acid undergoes a series of reactions, releasing two molecules of CO2 and regenerating oxaloacetate.

The cycle then restarts as oxaloacetate reacts with another acetyl-CoA molecule. ***As a result, each cycle produces two molecules of ATP and CO2, while the oxaloacetate is reused to form citrate*** (Berovic & Legisa, 2007)***.***

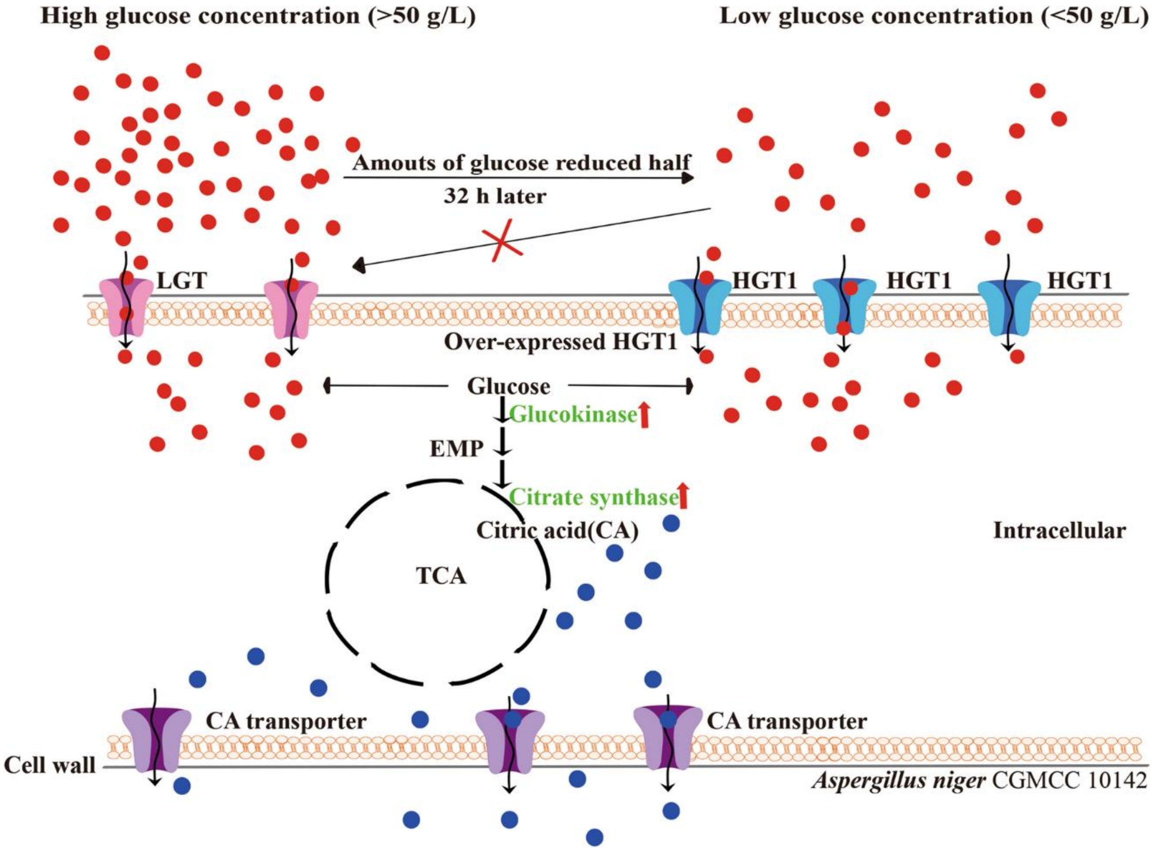


Figure 2. Krebs cycle (TCA) in *Aspergillus niger*, conversion of glucose in citric acid.

The global citric acid market reached a volume of 3.0 million tons in 2024. Looking ahead, the market is projected to grow to 3.7 million tons by 2033, with a compound annual growth rate (CAGR) of 2.5% from 2025 to 2033. The increasing use of citric acid as a preservative, flavor enhancer, and stabilizer in a broad range of processed foods and beverages, along with the rising demand for convenience foods and advancements in biotechnology, are the primary drivers of this market expansion. This growth highlights the diverse applications of citric acid across numerous industries. (Source: https://www.imarcgroup.com/citric-acid-manufacturing-plant)

2. RAW MATERIAL DATA

Currently, 99% of CA production globally is achieved through fermentation. The filamentous fungus *Aspergillus niger* is a preferred and highly effective producer of CA. The bio-production of CA typically involves three main steps: preparation and inoculation of the fermentation broth, fermentation, and the recovery/purification of the product, as illustrated in Fig. 3. The three commonly used methods for CA bio-production are submerged fermentation (SmF), surface fermentation (SF), and solid-state fermentation (SSF), as shown in Table 1. A variety of substrates can be utilized for efficient and cost-effective CA production, depending on the type of fermentation method employed (Singh Dhillon, Kaur Brar, Verma, & Tyagi, 2011).

Fermentation type	Fermented by-product	Citric acid yiled	Microorganism	Fermentation parameters
SF	Turnip whey	27-47 /sugar consumed	*A. niger* NCIM 595	28 °C/9-12 days
	Cotton waste	16.7 g/L	*A. niger ATCC 9142*	30 °C/7 days
	Brewery waste	78.5 /sugar consumed	*A. niger* ATCC 9142	30 °C/14 days
	Sweet potato starch hydrolysate	45.90 g/L	*A. niger* NIIB-A6	30 °C/11 days
SmF	Cane molasses	114 g/L	*A. niger* GCMC 7	30 °C/7 days
	Corn cobs	603.5 g/kg dry mass	*A. niger* NRRL 2001	30 °C/3 days
	Date syrup	50 %	*A. niger* ATCC 9142	30 °C
	Blackstrap molasses	31.1 g/L	*A. niger* GCB 75	30 °C/5 days
	Coconut oil	99.6 /fatty acids	*C. lipolytica*	28 °C/10 days
	Glycerol containing waste	55.7 %	*Y. lipolytica* A-101-1.22	7 days
	Rapeseed oil	66.6 g/L	*Y. lipolytica* N1	4 days
	Olive mill waste	28.88 g/L	*Y. lipolytica*	28 °C
	Crude glycerol	119 /fatty acids	*Y. lipolytica* YB-423	10 days
	Orange peel	53%	*A. niger* CECT 2090	30 °C
	Sphagnum peat moss-	354 g/kg dry mass	*A. niger* NRRL 567	35 °C/5 days
SSF	Apple pommace	124 g/kg dry mass	*A. niger* BC-1	30 °C
	Pinapple waste	51.5/sugar consumed	*A. niger* DS-1	30 °C/8 days

	Corn husk	259 g/kg dry mass	*A. niger* NRRL 2001	30 °C/5 days
	Coffee husk	150 g/kg dry mass	*A. niger* CFTRI 30	3 days
	Grape pommace	60 /sugar consumed	*A. niger* NRRL 567	4 days
	Banana peels	180 g/L	*A. niger* MTCC 282	28 °C/3 days

Table 1. Citric acid production *via* fermentation techiques utilizing by-products as substrates (Singh Dhillon et al., 2011).

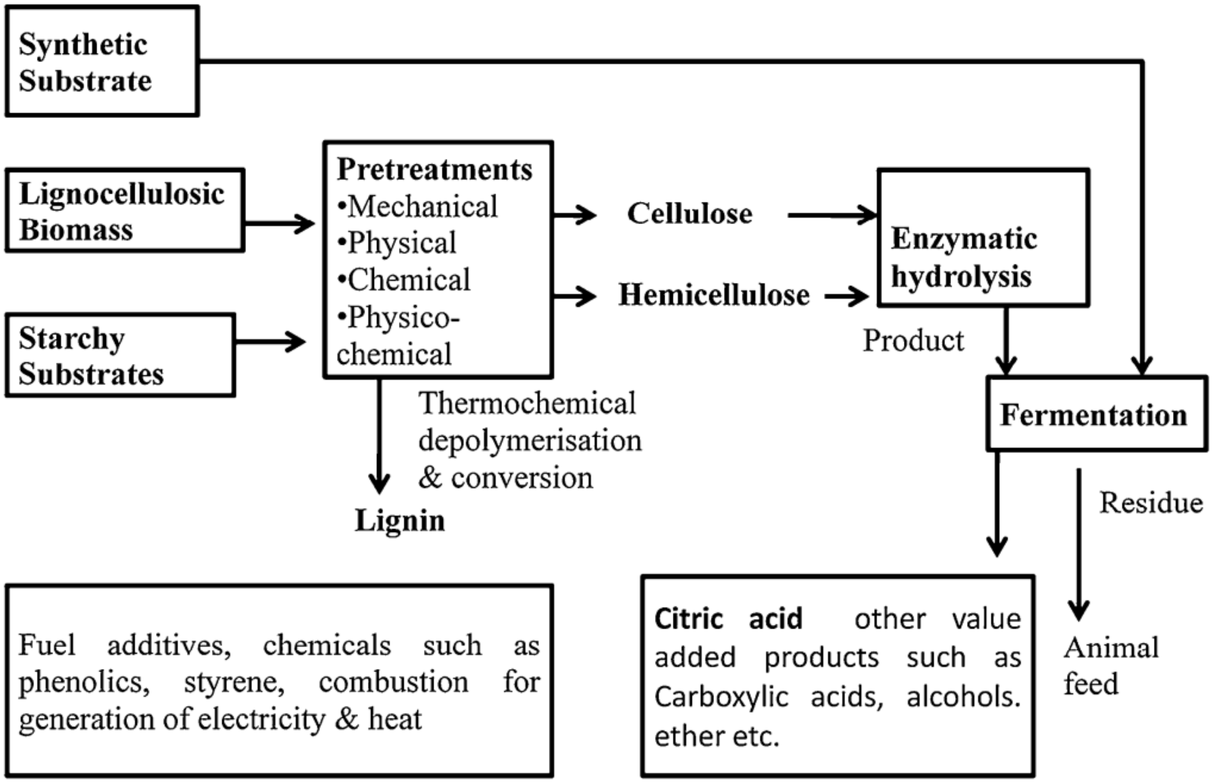


Figure 3. Flowchart showing the citric acid production process from different substrates

The SF process, which uses a liquid substrate, is one of the oldest methods for citric acid production, accounting for 5–10% of global supply. It remains in use due to its low investment, simple technology, and low energy requirements, despite higher labor costs compared to submerged fermentation. The process involves trays made of aluminum or stainless steel, arranged in racks, where aeration is provided by sterile air circulation for temperature regulation and gas exchange. Molasses, containing 15–20% sucrose, is acidified, heated, and treated with potassium hexacyanoferrate to remove trace metals and inhibit fungal growth. Inoculation occurs by adding conidia either as a suspension or aerosol. The temperature is maintained at 30°C, and within 24 hours, mycelium begins to form. As ammonium is absorbed, the pH drops to 2.0, and after 30 hours, the idiophase begins. The fermentation lasts 8–14 days, during which citric acid is produced, and mycelium forms a dense layer on the substrate (Berovic & Legisa, 2007).

The SSF process utilizing solid substrates, such as fibrous residues from apple, grape pomace, wheat bran, rice starch byproducts, and residual pulps from starch production (e.g., potato and sweet potato), is another approach to citric acid production. This method is based on traditional koji process techniques, with *Aspergillus niger* strains showing less sensitivity to trace elements compared to liquid substrate surface fermentation or submerged fermentation processes. In this process, the solid substrate is hydrated to achieve a water content of 65–70%. Following the removal of excess water, the substrate is subjected to steaming for sterilization. After sterilization, a sterile starch paste is inoculated by either spraying *Aspergillus niger* conidia as an aerosol or adding them as a liquid suspension onto the substrate surface. The substrate is maintained at a pH of 5–5.5 and incubated at temperatures ranging from 28°C to 30°C. The fungal growth can be accelerated by adding α-amylase, although *Aspergillus niger* naturally produces its own α-amylase to hydrolyze starch. During the fermentation, the pH of the substrate drops below 2 due to the production of citric acid. The solid-state fermentation process typically lasts 5–8 days, after which the entire mass is extracted with hot water to recover the citric acid. In some cases, mechanical processes may also be employed to further extract citric acid from the fungal biomass. For example, the use of cane bagasse as the substrate for solid-state fermentation yielded citric acid in 6 days. ***In 1990, the global production of citric acid via solid-state fermentation was approximately 350,000 tons***.

The SmF process is a viable alternative to surface fermentation, offering advantages like lower investment (2.5 times less), 25% reduced total costs, and improved process control and sterility. However, it requires more energy and specialized personnel. Key factors influencing high-yield citric acid production in submerged fermentation are the quality of stainless steel used for bioreactors, mycelium structure, and oxygen transfer efficiency. Commonly used bioreactors include stirred tank and airlift reactors. Immobile Aspergillus niger cells on carriers such as glass or polyurethane foams have been explored for enhanced fermentation. Bioreactors must be acid-resistant to avoid inhibition by leached metals at low pH. Substrates like molasses, starch hydrolysates, and waste oils are used, with carbon concentrations between 14–27% yielding optimal results (Berovic & Legisa, 2007).

Recent advancements in SSF have highlighted its potential as a viable alternative for industrial-scale CA production. To enhance the feasibility of SSF-based citric acid bioproduction, research has primarily focused on the utilization of agro-industrial residues as substrates. ***These residues are abundant, cost-effective, rich in carbohydrates, and contain essential nutrients***. Furthermore, they facilitate the growth of filamentous fungi, such as *Aspergillus niger*, which can effectively penetrate solid substrates through the turgor pressure at the mycelial tips.***The application of these low-cost agro-industrial by-products in SSF not only improves the economic efficiency of citric acid production but also addresses environmental concerns related to waste disposal***. By repurposing such residues, industries can achieve dual benefits of sustainable production and waste management (Singh Dhillon et al., 2011).

The selection of an appropriate substrate for SSF depends on several factors, primarily its cost, nutrient composition, and availability. ***Agro-industrial residues are particularly suited for SSF processes as they inherently supply the necessary nutrients for microbial proliferation.*** Common substrates derived from agro-industrial residues include beet and sugarcane molasses, cellulose, lipids, whey, fruit pomaces, starch-based materials, sweet potatoes, cassava, seaweed, and glycerol. These by-products are particularly advantageous in solid-state fermentation due to their availability, low cost, and ability to support microbial growth. These materials represent a sustainable and economical approach for large-scale citric acid production.

Cane and beet molasses are especially prominent as raw materials because of their affordability and high carbohydrate content, primarily in the form of sucrose, glucose, and fructose. However, their variable chemical composition can pose challenges for citric acid biosynthesis. To improve fermentation efficiency, molasses is often pretreated using processes like clarification, acid or alkaline treatment, and fractionation, which enhance its suitability as a substrate and improve overall yields.

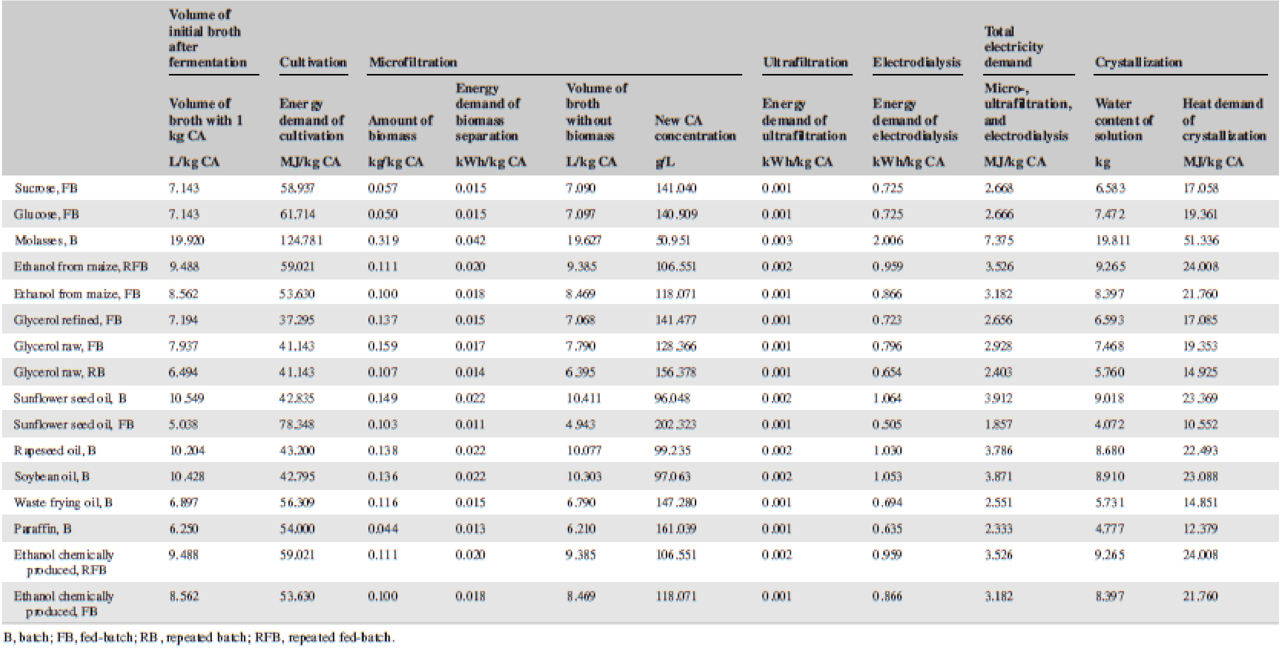
Sucrose is another ideal carbon source for citric acid production due to its low molecular weight, which allows efficient transport into microbial cells, and its rapid breakdown by enzymes activated in acidic conditions. High-purity sucrose enables precise control over the fermentation process and can achieve high yields of citric acid. However, the high cost of pure sucrose often outweighs the value of the final product, making it less suitable for large-scale industrial use (Książek, 2023).

3. LIFE CYCLE ASSESSMENT OF CITRIC ACID PRODUCTION

The life cycle assessment (LCA) of CA production involves evaluating various production processes' environmental, social, and economic impacts. These processes include substrate conversion, downstream processing (DSP), and energy demand during fermentation and product recovery. Becker et al. (2018) conducted a comprehensive life cycle assessment (LCA) of citric acid (CA) production, utilizing the software GaBi8© to evaluate the environmental, social, and economic performance of various bioprocesses. The analysis was centred around producing 1 kg of CA as the functional unit, employing the microorganism *Yarrowia lipolytica* in the cultivation process. This yeast was chosen for its potential to convert diverse substrates into CA under varying conditions efficiently. The analysis covered multiple substrate-based production methods and considered parameters such as global warming potential (GWP), human toxicity potential (HTP), and economic viability (Becker et al., 2020).

Fermentation Process

Substrates and nutrients are converted into CA in the fermentation process. A neutralizer is added to the solution, increasing the final volume compared to the starting compound. The fermentation broth contains CA and the neutralizer, which serves as the input for downstream processing (DSP). Data were normalized to the functional unit of 1 kg CA by dividing by the amount of CA produced. The energy demand for mixing and aerating reactors was set at 12 kW/m³, based on laboratory-scale reactors. The volume of fermentation broth required to produce 1 kg CA was calculated using the CA concentration in the process data (Figure 4).



	Volume of initial broth after fermentation	Cultivation	Microfiltration				Ultrafiltration	Electrodialysis	Total electricity demand	Crystallization	
	Volume of broth with 1 kg CA	Energy demand of cultivation	Amount of biomass	Energy demand of biomass separation	Volume of broth without biomass	New CA concentration	Energy demand of ultrafiltration	Energy demand of electrodialysis	Micro-, ultrafiltration, and electrodialysis	Water content of solution	Heat demand of crystallization
	L/kg CA	MJ/kg CA	kg/kg CA	kWh/kg CA	L/kg CA	g/L	kWh/kg CA	kWh/kg CA	MJ/kg CA	kg	MJ/kg CA
Sucrose, FB	7.143	58.937	0.057	0.015	7.090	141.040	0.001	0.725	2.668	6.583	17.058
Glucose, FB	7.143	61.714	0.050	0.015	7.097	140.909	0.001	0.725	2.666	7.472	19.361
Molasses, B	19.920	124.781	0.319	0.042	19.627	50.951	0.003	2.006	7.375	19.811	51.336
Ethanol from maize, RFB	9.488	59.021	0.111	0.020	9.385	106.551	0.002	0.959	3.526	9.265	24.008
Ethanol from maize, FB	8.562	53.630	0.100	0.018	8.469	118.071	0.001	0.866	3.182	8.397	21.760
Glycerol refined, FB	7.194	37.295	0.137	0.015	7.068	141.477	0.001	0.723	2.656	6.593	17.085
Glycerol raw, FB	7.937	41.143	0.159	0.017	7.790	128.366	0.001	0.796	2.928	7.468	19.353
Glycerol raw, RB	6.494	41.143	0.107	0.014	6.395	156.378	0.001	0.654	2.403	5.760	14.925
Sunflower seed oil, B	10.549	42.835	0.149	0.022	10.411	96.048	0.002	1.064	3.912	9.018	23.369
Sunflower seed oil, FB	5.038	78.348	0.103	0.011	4.943	202.323	0.001	0.505	1.857	4.072	10.552
Rapeseed oil, B	10.204	43.200	0.138	0.022	10.077	99.235	0.002	1.030	3.786	8.680	22.493
Soybean oil, B	10.428	42.795	0.136	0.022	10.303	97.063	0.002	1.053	3.871	8.910	23.088
Waste frying oil, B	6.897	56.309	0.116	0.015	6.790	147.280	0.001	0.694	2.551	5.731	14.851
Paraffin, B	6.250	54.000	0.044	0.013	6.210	161.039	0.001	0.635	2.333	4.777	12.379
Ethanol chemically produced, RFB	9.488	59.021	0.111	0.020	9.385	106.551	0.002	0.959	3.526	9.265	24.008
Ethanol chemically produced, FB	8.562	53.630	0.100	0.018	8.469	118.071	0.001	0.866	3.182	8.397	21.760

B, batch; FB, fed-batch; RB, repeated batch; RFB, repeated fed-batch.

Figure 4. Energy demand of cultivation and down-stream processing

Downstream Processing (DSP)

DSP begins with biomass separation using microfiltration. Since specific energy data for filtering *Yarrowia lipolytica* was unavailable, the energy demand was approximated using data for microalgae filtration, averaging 2.125 kWh/m³. The biomass volume was calculated by considering broth volume and biomass concentration, and this amount was subtracted from the broth volume. Subsequently, proteins were removed *via* ultrafiltration, with an energy demand of 0.17 kWh/L, averaged from clean and fouled membrane data. Electrodialysis followed, using cation exchange and bipolar membranes to remove Na^- ions. Based on experimental results, the energy demand for this step was set at 0.102 kWh/L. The final steps involved condensing, crystallizing, and drying the CA solution. Due to limited literature data, energy demand was estimated using the heat capacity of water and the heat of vaporization. This calculation assumed heating the liquid from 293.15 K to 373.15 K and evaporating the remaining water, excluding heat losses.

Environmental Assessment

GWP and HTP were key indicators of environmental impact. Substrates produced via energy-intensive processes or those with low CA concentration and long cultivation times had higher environmental burdens. For example:

- Glycerol-based processes demonstrated high CA concentrations (154 g/L) and short cultivation times (147 hours), resulting in the best environmental performance.

- Sunflower oil-fed batch processes achieved the highest CA concentration (198.5 g/L) but required long cultivation times (360 hours), ranking lower in environmental performance.
- Substrates like molasses and chemically produced ethanol ranked poorly due to low CA concentrations and higher energy demands.

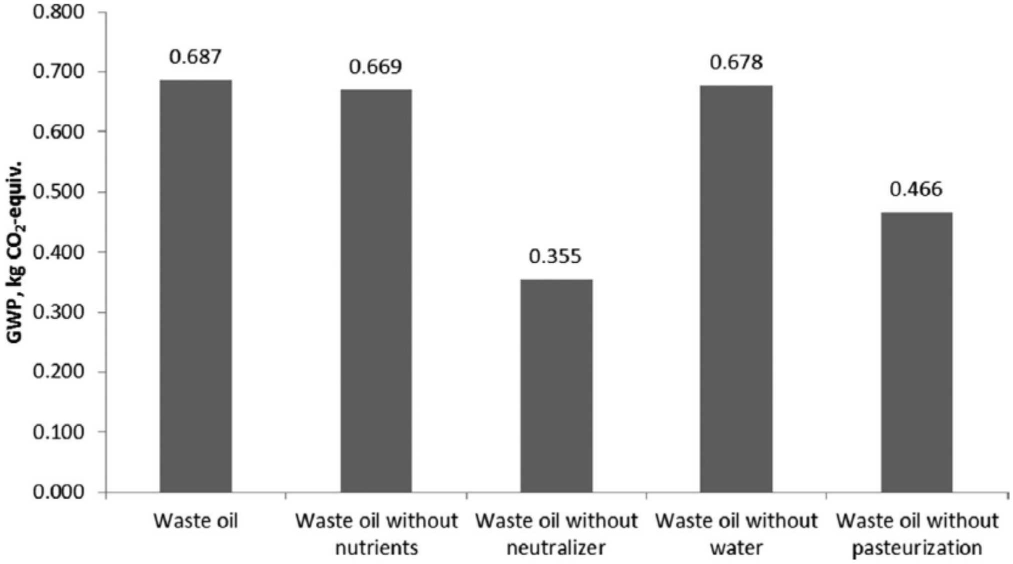
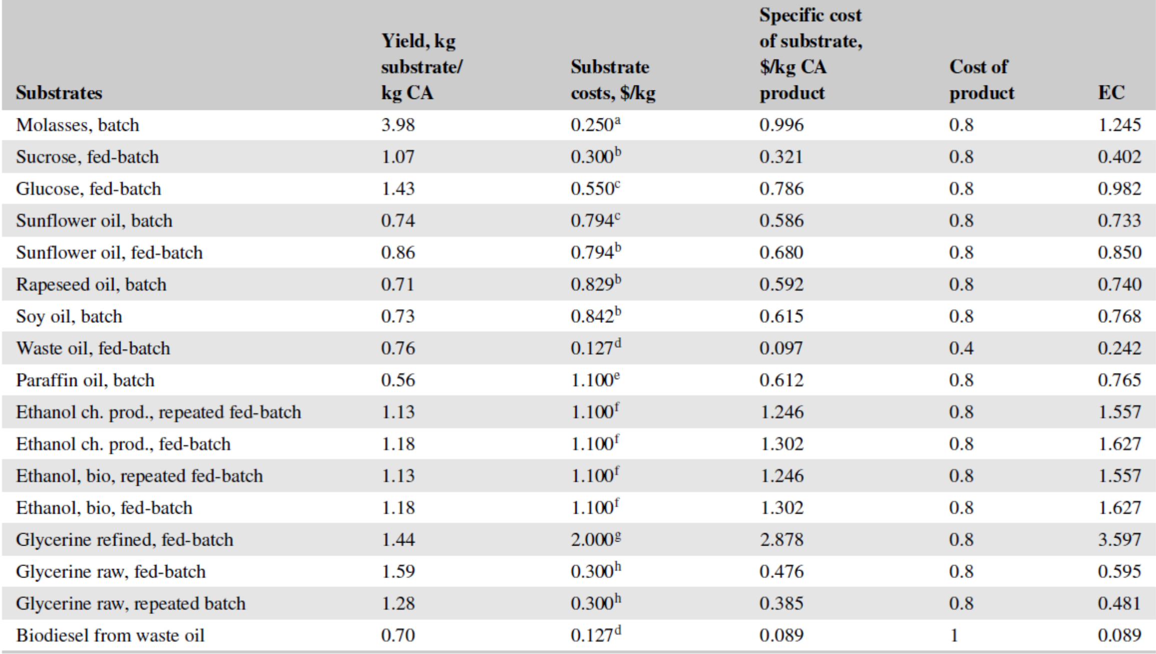


Figure 5. Environmental impacts of four alternative processes using waste oil

Economic and Social Indicators

The economic assessment highlighted that CA production from waste oil offered the best performance, costing $0.4/kg. However, this is less economically competitive than waste frying oil utilization for biodiesel production, which requires a CA product price of $1.1/kg to be viable.



Substrates	Yield, kg substrate/ kg CA	Substrate costs, $/kg	Specific cost of substrate, $/kg CA product	Cost of product	EC
Molasses, batch	3.98	0.250[a]	0.996	0.8	1.245
Sucrose, fed-batch	1.07	0.300[b]	0.321	0.8	0.402
Glucose, fed-batch	1.43	0.550[c]	0.786	0.8	0.982
Sunflower oil, batch	0.74	0.794[c]	0.586	0.8	0.733
Sunflower oil, fed-batch	0.86	0.794[b]	0.680	0.8	0.850
Rapeseed oil, batch	0.71	0.829[b]	0.592	0.8	0.740
Soy oil, batch	0.73	0.842[b]	0.615	0.8	0.768
Waste oil, fed-batch	0.76	0.127[d]	0.097	0.4	0.242
Paraffin oil, batch	0.56	1.100[e]	0.612	0.8	0.765
Ethanol ch. prod., repeated fed-batch	1.13	1.100[f]	1.246	0.8	1.557
Ethanol ch. prod., fed-batch	1.18	1.100[f]	1.302	0.8	1.627
Ethanol, bio, repeated fed-batch	1.13	1.100[f]	1.246	0.8	1.557
Ethanol, bio, fed-batch	1.18	1.100[f]	1.302	0.8	1.627
Glycerine refined, fed-batch	1.44	2.000[g]	2.878	0.8	3.597
Glycerine raw, fed-batch	1.59	0.300[h]	0.476	0.8	0.595
Glycerine raw, repeated batch	1.28	0.300[h]	0.385	0.8	0.481
Biodiesel from waste oil	0.70	0.127[d]	0.089	1	0.089

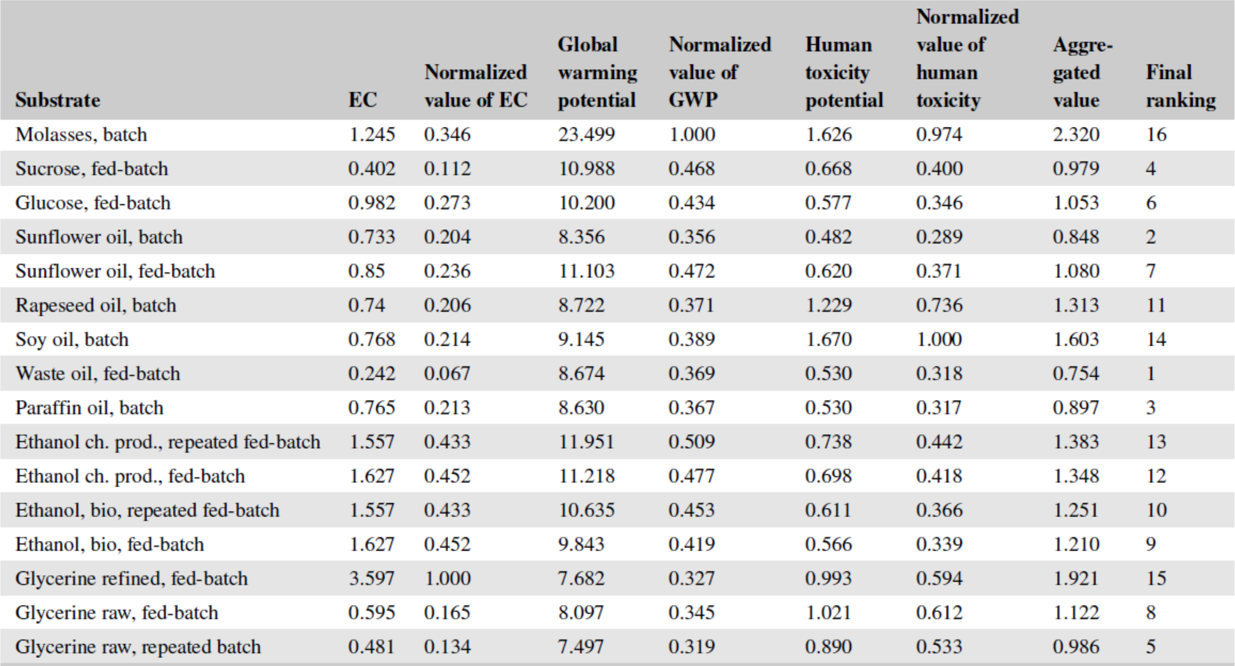
Figure 6. Data and results of economic indicators

Social indicators, particularly HTP, showed that waste oil-based CA production ranked third. The sunflower oil batch process ranked highest due to moderate CA concentration and competitive cultivation times. Processes using soybean oil and refined glycerol had the lowest sustainability due to emissions of toxic compounds such as arsenic, selenium, and benzene.

Results and Aggregation of Substrate Performance

The life cycle assessment (LCA) conducted by Becker et al. (2018) analyzed the environmental, economic, and human health impacts of citric acid (CA) production using various substrates. The study included an evaluation of 16 substrates based on three key metrics: Economic Cost (EC), Global Warming Potential (GWP), and Human Toxicity Potential (HTP). Normalized values were presented for each metric, with aggregated rankings for the substrates and their corresponding process configurations.

The lowest normalized EC value (0.067) was achieved using waste oil in a fed-batch process, highlighting its economic advantage. In contrast, refined glycerine in a fed-batch process had the highest EC value (1.000), reflecting the high production costs. For environmental sustainability, the waste oil-fed batch process also exhibited the lowest normalized GWP value (0.367), making it the most environmentally sustainable option. Molasses in a batch process exhibited the highest GWP (1.000), attributed to energy-intensive processing and substrate production. Regarding human health impacts, waste oil-fed batch and paraffin oil batch processes tied for the lowest HTP values (0.318 and 0.317, respectively), demonstrating lower health-related risks. Soy oil in a batch process showed the highest HTP value (1.000), indicating significant potential for adverse human health impacts.



Substrate	EC	Normalized value of EC	Global warming potential	Normalized value of GWP	Human toxicity potential	Normalized value of human toxicity	Aggre-gated value	Final ranking
Molasses, batch	1.245	0.346	23.499	1.000	1.626	0.974	2.320	16
Sucrose, fed-batch	0.402	0.112	10.988	0.468	0.668	0.400	0.979	4
Glucose, fed-batch	0.982	0.273	10.200	0.434	0.577	0.346	1.053	6
Sunflower oil, batch	0.733	0.204	8.356	0.356	0.482	0.289	0.848	2
Sunflower oil, fed-batch	0.85	0.236	11.103	0.472	0.620	0.371	1.080	7
Rapeseed oil, batch	0.74	0.206	8.722	0.371	1.229	0.736	1.313	11
Soy oil, batch	0.768	0.214	9.145	0.389	1.670	1.000	1.603	14
Waste oil, fed-batch	0.242	0.067	8.674	0.369	0.530	0.318	0.754	1
Paraffin oil, batch	0.765	0.213	8.630	0.367	0.530	0.317	0.897	3
Ethanol ch. prod., repeated fed-batch	1.557	0.433	11.951	0.509	0.738	0.442	1.383	13
Ethanol ch. prod., fed-batch	1.627	0.452	11.218	0.477	0.698	0.418	1.348	12
Ethanol, bio, repeated fed-batch	1.557	0.433	10.635	0.453	0.611	0.366	1.251	10
Ethanol, bio, fed-batch	1.627	0.452	9.843	0.419	0.566	0.339	1.210	9
Glycerine refined, fed-batch	3.597	1.000	7.682	0.327	0.993	0.594	1.921	15
Glycerine raw, fed-batch	0.595	0.165	8.097	0.345	1.021	0.612	1.122	8
Glycerine raw, repeated batch	0.481	0.134	7.497	0.319	0.890	0.533	0.986	5

Figure 7. Results and aggregation

In terms of aggregated performance, waste oil in a fed-batch process ranked first overall, driven by its low EC (0.067), GWP (0.367), and HTP (0.318) values, highlighting its viability for sustainable and cost-effective CA production. Sunflower oil in a batch process and paraffin oil in a batch process ranked second and third, respectively, due to their balanced performance across all metrics. Molasses in a batch process ranked last (16th), primarily due to its high GWP (1.000) and HTP (1.000) scores, despite moderate economic costs. Refined glycerine in a fed-batch process ranked 15th, reflecting its high EC (1.000) and relatively poor performance in HTP.

4. CASE EXAMPLES OF COMPANIES THAT TRANSITIONED TO CIRCULAR FERMENTATION FOR CITRIC ACID PRODUCTION.

CitriBel

- Background: Citribel is a leading company specializing in the production of citric acid, a versatile organic acid used in a variety of industries, including food, pharmaceuticals, and detergents. The company has embraced a circular production model, utilizing fermentation and innovative waste management practices to minimize environmental impact.
- Transition Details: The company pioneered the use of fermentation to produce mycoprotein, which is cultivated from Fusarium venenatum, a type of fungus, and blended into various meat substitute products.

- Impact: Citribel's circular approach to citric acid production significantly reduces environmental impact by maximizing resource utilization and minimizing waste. By adopting renewable raw materials and optimizing energy usage, the company has decreased its reliance on fossil fuels and reduced greenhouse gas emissions.
- Web site: https://www.citribel.com/products-services/citric-acid/

Citribel sets itself apart by utilizing a unique surface fermentation process to produce premium citric acid anhydrous and citric acid monohydrate. This distinctive method not only ensures the purity of the final product but also highlights the company's commitment to delivering high-quality circular products. As a leading citric acid producer, customers can trust Citribel's citric acid for its excellence in both production and performance. Citribel's citric acid is produced sustainably using residual flows from the sugar chain as raw material. This commitment to circularity not only makes the production process eco-friendly, it also results in high-quality circular products. Choosing Citribel's citric acid means opting for an environmentally conscious solution that aligns with the principles of sustainability.

Citribel fungus grows into a biomass (known as mycelium) during our unique surface fermentation process, while simultaneously converting the sugar in the molasses into citric acid. We monitor and adjust an extensive variety of factors in 184 fermentation chambers (such as temperature, humidity, surface tension, etc.). After a few days, when all the sugar has been converted into citric acid, the mycelium (Citrocell®) and the crude citric acid solution are carefully separated. Next, the citric acid is transformed into pure, white crystals in our refinery. Here, it undergoes a reaction with lime milk, forming a calcium salt precipitate. The filtrate is pumped to our feed & agro business facility, giving origin to our Nutribond® and Citrofert®.

Weifang Ensign Industry Co., Ltd.

- Background: Established in 1997 and headquartered in China, Weifang Ensign Industry is a prominent manufacturer of citric acid and alcohol products. The company specializes in producing citric acid monohydrate, sodium citrate, citric acid anhydrous, and high-grade alcohol.
- Transition Details: Weifang Ensign utilizes starch-based raw materials, such as sweet potato, tapioca, and corn, to manufacture its products through advanced biological fermentation techniques. The company employs a circular production model, focusing on resource efficiency and waste minimization. By integrating modern equipment and leveraging strong technical expertise, Weifang Ensign has optimized the fermentation process to convert renewable raw materials into high-quality citric acid products.
- Website: http://www.ensignworld.com/

Weifang Ensign's circular production system significantly reduces environmental impact by utilizing renewable agricultural inputs and minimizing waste. The company's innovative approach not only ensures the efficient use of resources but also contributes to reducing carbon emissions and supporting a circular economy. Its commitment to sustainability reflects its leadership in environmentally responsible citric acid production.

5. ANALYTICAL TOOLS FOR CITRIC ACID PRODUCTION AND REGULATORY GUIDELINES

Citric acid is a key organic acid widely used in food, pharmaceutical, and industrial applications. Ensuring the quality and efficiency of its production requires advanced analytical techniques to monitor processes, optimize yields, and maintain product purity. These tools enable precise quantification of citric acid, detection of impurities, and real-time assessment of fermentation conditions, making them indispensable in both research and industrial settings. Below is an overview of the primary analytical methods used in citric acid production.

A. Chromatographic Techniques

- **High-Performance Liquid Chromatography (HPLC):**
    - Widely used to quantify citric acid concentration in fermentation broths.
    - Equipped with detectors such as UV-Vis, Refractive Index (RI), or Diode Array Detectors (DAD) for accurate measurement.
- **Gas Chromatography (GC):**
    - Used for volatile compound analysis in citric acid production.
    - Coupled with Mass Spectrometry (GC-MS) for detailed profiling of impurities or by-products.
- **HPLC-MS or LC-MS/MS:**
    - Combines liquid chromatography with mass spectrometry for detailed identification and quantification of citric acid
    - Detects trace impurities and minor fermentation by-products

B. Spectroscopic Techniques

- **UV-Vis Spectrophotometry:**
    - Assesses citric acid concentration by analyzing its absorbance at specific wavelengths.
    - Simple, fast, and cost-effective for routine analysis.
- **Fourier Transform Infrared (FTIR) Spectroscopy:**
    - Identifies functional groups and confirms citric acid's chemical structure.

  - Useful for quality control and verifying product purity.
- **Nuclear Magnetic Resonance (NMR):**
  - Determines molecular structure and assesses the purity of citric acid.
  - Provides insights into metabolic intermediates during fermentation.

C. Thermal Analysis

- **Differential Scanning Calorimetry (DSC):**
  - Assesses the thermal stability of citric acid.
  - Helps evaluate the quality of solid-state fermentation products.

REGULATORY GUIDELINES EXAMPLES:

The Environmental Protection Agency (EPA) has evaluated citric acid, the pesticide active (and inert) ingredient involved in registration review Case #4024 (Docket ID EPA-HQ-OPP-2020-0558 and EPA-HQ-OPP-2008-0855, available at www.regulations.gov). Based on the information that follows, the EPA has determined that citric acid is not anticipated to produce an effect in humans, or other organisms, similar to an effect produced by naturally occurring estrogen, androgen, or thyroid hormones because it is a naturally occurring ingredient present in the environment and in foods, has limited to no toxic effects, and nothing in the available data indicates that citric acid would be likely to produce such an effect. Therefore, pursuant to the Federal Food, Drug, and Cosmetic Act (FFDCA) Section 408(p)(4), the EPA hereby exempts the current uses of citric acid from the requirements of the Endocrine Disruptor Screening Program (EDSP).

Regulation examples:

1.Commission regulation (eu) 2020/351

- ➢ The use of citric acid (E 330) in cocoa and chocolate products

2. Commission Regulation (EU) No 231/2012

- ➢ Specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008, E 330 CITRIC ACID

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