Supplementary Information (SI) for RSC Sustainability.

Advancing Bioethanol: Exploring Feedstock Diversity, Production Pathways, and Environmental Implications

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Supplementary Information

Table S1. Potential feedstocks for bioethanol synthesis.

1st Generation	Sugar-based	Sugarcane, Sugar beet, Sweet sorghum (stalk juice and grains), Sugar Maple, Tropical sugar crops (sugar palm and nipa palm), Fruit based sugars, Honey, Agave nectar, Cactus pear, Carob pods			[1], [2], [3], [4], [5], [6], [7], [8], [9]
	Starch-based	Cereals & Grains	Corn (Maize), Wheat, Barley, Rice, Sorghum grains, Millets, Oats, Rye		[10], [11], [12], [13]
		Beans & Lentils	Chickpeas (Garbanzo beans), Lentils, Faba Beans (broad beans), Peas (*Pisum sativum*)		
		Nuts	Chestnuts kernels, Acorn nuts		
		Starchy Vegetables	Potato tuber, Sweet potato tuber, Cassava roots, Taro corms, Jerusalem artichoke tuber		
2nd Generation	Lignocellulosic biomass	Edible oil crop cake	Soybeans, Rapeseeds (Canola), Sunflower, Palm, Coconut, Peanut (Groundnut), Cottonseed, Sesame, Safflower		[14]
		Virgin wood	Poplar (*Populus spp.*), Eucalyptus (*Eucalyptus spp.*), Pine (*Pinus spp.*), Douglas Fir (*Pseudotsuga spp.*), Acacia (*Acacia spp.*), Black Locust (*Robinia pseudoacacia*), Cottonwood (*Populus deltoides*), Sweetgum (*Liquidambar styraciflua*), Birch (*Betula spp.*)		[15], [16], [17], [18], [19]
		Forest residue	Primary forest residue	Branches, Stumps, Treetops, Bark, Sawdust	[20]
			Secondary forest residue	Bark, Sawdust, Sawmill slabs, Wood chips	
		Agricultural residue	Corn stover, Wheat straw, Rice straw, Sugarcane bagasse, Sorghum stalk, Corn husk, Olive Pomace, Barley straw, Oats straw, Cotton stalks		[21], [22], [23]
		Dedicated energy crops	Energy cane (*Saccharum spp.* hybrid) fibers, Jatropha (*Jatropha curcas*) cake, Camelina (*Camelina sativa*) cake, Miscanthus (*Miscanthus spp.*), Switchgrass (*Panicum virgatum*), Willow (*Salix spp.*), Giant reed (*Arundo donax*), Napier Grass (*Pennisetum purpureum*), Kenaf (*Hibiscus cannabinus*)		[24], [25], [26], [27], [28]

		Water plants	Water hyacinth, Duckweed (*Lemna spp.* and others), Seagrass (*Zostera spp.*)	[29], [30], [31]
	Chitin/Chitosan	Outer shells of arthropods, such as insects and crustaceans		[32]
	Waste stream	Municipal solid waste	Food waste, Yard waste, Paper waste, Kitchen waste, Sewage sludge	[33], [34], [35], [36]
		Organic industrial waste	Dry distiller grains#, Glycerol, Lignin, Brewery waste	[37], [38], [39], [40]
3rd Generation*	Microalgae	Diatoms	*Phaeodactylum tricornutum, Thalassiosira pseudonana, Skeletonema costatum*	[41], [42]
		Dinoflagellates	*Crypthecodinium cohnii, Karenia spp., Prorocentrum spp.*	[43], [44], [45]
		Cyanobacteria (Blue-Green Algae)	*Nostoc muscorum, Nostoc sp., Arthrospira platensis*	[46], [47], [48]
	Macroalgae (seaweed)	Brown algae	*Laminaria japonica, Saccharina japonica, Macrocystis pyrifera*	[49], [50], [51]
		Red algae	*Gelidium amansii, Kappaphycus alvarezii, Gracilaria verrucosa, Eucheuma cottonii*	[52], [53]
		Green algae	*Ulva fasciata Delile, Ulva lactuca, Ulva intestinalis*	[54], [55], [56]
4th Generation	GM plants and crops	GM switchgrass, GM Populus, GM Maize, GM Rice straw (Cesa7 mutant), GM sugarcane, GM Alfalfa (*Medicago sativa*)		[57], [58], [59], [60], [61], [62]
	GM algae	GM microalgae (*Chlamydomonas reinhardtii*),		[63]
	Engineered Microbes	*C. ragsdalei* (DSM 15248), *Scheffersomyces stipitis* SP2-18, Genetically modified strains of *S. cerevisiae* (D452-2)		[64], [65], [66]
	Artificial Photosynthetic systems	Genetically modified cyanobacterial strain *Synechocystis sp*. PCC 6803		[67]

*Mostly, algae have high lipid content and are used to produce biodiesel. The carb-rich cell wall is used to produce bioethanol.
#considered as second-generation because it is a residual product rather than a primary food source.

Table S2. Comparison of microalgae and macroalgae (third-generation biomass) based on morphological characteristics and other key parameters.

Characteristics	Microalgae	Macroalgae (seaweed)	Reference
Size	Unicellular or colonial organisms, typically less than 0.1 mm in size	Multicellular and macroscopic algae, typical size ranging from a few centimeters to several meters in length.	[68], [69]
Habitat	Freshwater, marine water, and sometimes moist terrestrial habitats	Predominantly marine environments, attached to substrates or free-floating	[68], [70], [71], [72]
Photosynthetic Pigments	Chlorophyll a and various accessory pigments like chlorophyll b, c, and carotenoids	Chlorophyll a, b, and accessory pigments like phycobilin (in red and blue-green algae) and fucoxanthin (in brown algae).	[73], [74], [75]
Classifications	Classified into groups such as diatoms, dinoflagellates, and cyanobacteria (blue-green algae)	Classified based on their color (brown, red, green) and structure (filamentous, leafy, encrusting).	[76], [77], [78]
Application	Typically studied for their lipid content for biofuel production, but some can also be used for ethanol production from their carbohydrates.	These are rich in polysaccharides like cellulose and alginate, which can be converted into fermentable sugars for bioethanol production.	[79], [80], [81]

Table S3. Fermentation classifications based on the reactor's operational mode.

Fermentation Type	Overview	Pros	Cons	References
Batch	Simultaneous initial feeding of all components (nutrients, microbes, and ingredients). Sugar and ethanol concentration changes over time, alongside pH and temperature fluctuations.	Low cost and contamination risk; Easy to sterilize and operate.	Lesser ethanol production due to low cell density; Downtime is long as emptying, vessel setup, cleaning, sterilization, and refilling take time.	[82], [83], [84]
Fed-Batch	Microbes inoculated in the media grow under batch regime by feeding on the substrate present. Later, the substrate is added incrementally throughout the fermentation.	Better control of pH, dissolved oxygen, and temperature; limited byproduct formation; Increased cell lifespan; maximum viable cells in the reactor at any time; Elevated concentration of ethanol.	Higher cost to control the process; Downtime is long as emptying, cleaning, sterilization, vessel setup, and refilling takes time.	[82], [83], [85]
Continuous	Carried out in plug flow reactor or single or series of stirred tank reactor. Fresh media continuously replaces used media (having ethanol, toxic metabolites, and low nutrient content).	Achieve greater ethanol yield, cost efficiency, and heightened productivity on a smaller scale; Effortlessly manageable and controllable; Time-saving benefits for tasks like emptying, cleaning, sterilization, refilling, and media adjustment.	Contamination risks can cause bioprocess failure; Complete sterilization and cleaning of the process line are necessary; fresh inoculum is needed; Cell aggregation can impede steady-state growth; and maintaining filamentous organisms in viscous, heterogeneous media is complex.	[82], [83], [86], [87], [88], [89]

Bioethanol as a Platform Chemical: Potential and Opportunities

Fuel and Energy Sector

Ethanol is commonly blended with gasoline to produce E10 (10% ethanol), E15 (15% ethanol), and E85 (85% ethanol) fuels [90]. These blends help reduce GHG emissions and improve octane ratings. Several nations, like the United States, India, Europe, and Brazil, have mandated the blending of bioethanol[91], [92], [93], [94]. Research is ongoing to develop advanced biofuels that combine ethanol with other renewable resources, potentially enhancing fuel properties and expanding usage [95]. Shurong Wang et al. [96] derived bio-oil from the pyrolysis of biomass, which was then blended with ethanol using the co-cracking method by engaging an HSZM-5 catalyst. At optimal conditions of 400°C and 2 MPa, this process yielded a high hydrocarbon content (99%) comprising C7-C10 aromatic hydrocarbons, toluene, xylene, and methyl-ethyl-benzenes. Ethanol can also be reformed to produce hydrogen, which can be used in fuel cells for clean energy applications [97]. This process offers a renewable pathway for hydrogen production, complementing other methods like water electrolysis [98]. Moreover, ethanol-derived jet fuel is an emerging area, offering a sustainable alternative to conventional aviation fuels [99].

Chemical, Healthcare, and Pharmaceutical Industry

Bioethanol can be suitably used as a chemical intermediate, eliminating the use of conventional ethanol and making a step forward towards sustainable and green synthesis of chemicals. For example, bioethanol can be dehydrated to produce ethylene [100], a key building block for numerous chemicals, including polyethylene (used in the manufacturing of bioplastics [101]), ethylene oxide (used in detergents and antifreeze), and ethylene glycol (used in antifreeze and polyester). Also, the oxidation of bioethanol produces acetic acid [102], a precursor for various chemicals, including vinyl acetate (used in adhesives and paints) and acetate esters (used as solvents). Bioethanol can be widely used as a solvent in industries such as pharmaceuticals, cosmetics, and food processing owing to its low toxicity and effective solvating properties. These are the niche markets that can pay better prices for bioethanol rather than fuel [103]. Other healthcare and sanitation applications of bioethanol are drug formulation (as it enhances solubility and stability of active ingredients), sanitizers, and disinfectants (due to its antimicrobial properties)

Food and Beverage Industry

Bioethanol is the primary alcohol produced by the fermentation of sugars in the production of alcoholic beverages like beer, wine, and spirits [104]. It is also used as a preservative in food products to inhibit microbial growth and extend shelf life [105]. Bioethanol is sometimes used as a carrier and solvent for flavors and colorants in a variety of food and beverage products [106].

Other Industrial Applications

Bioethanol serves as a solvent in the manufacturing of industrial coatings, adhesives, and paints, ensuring optimal viscosity and drying characteristics. It also serves as an effective solvent for industrial cleaning agents to remove grease, oil, and other contaminants from machinery and equipment. Bioethanol's excellent thermal properties make it suitable for use in cooling systems for high-performance computing and electronic devices [107]. Its low freezing point and high heat of vaporization offer advantages over traditional coolants [108].

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