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Microorganisms as hydrocarbon producers

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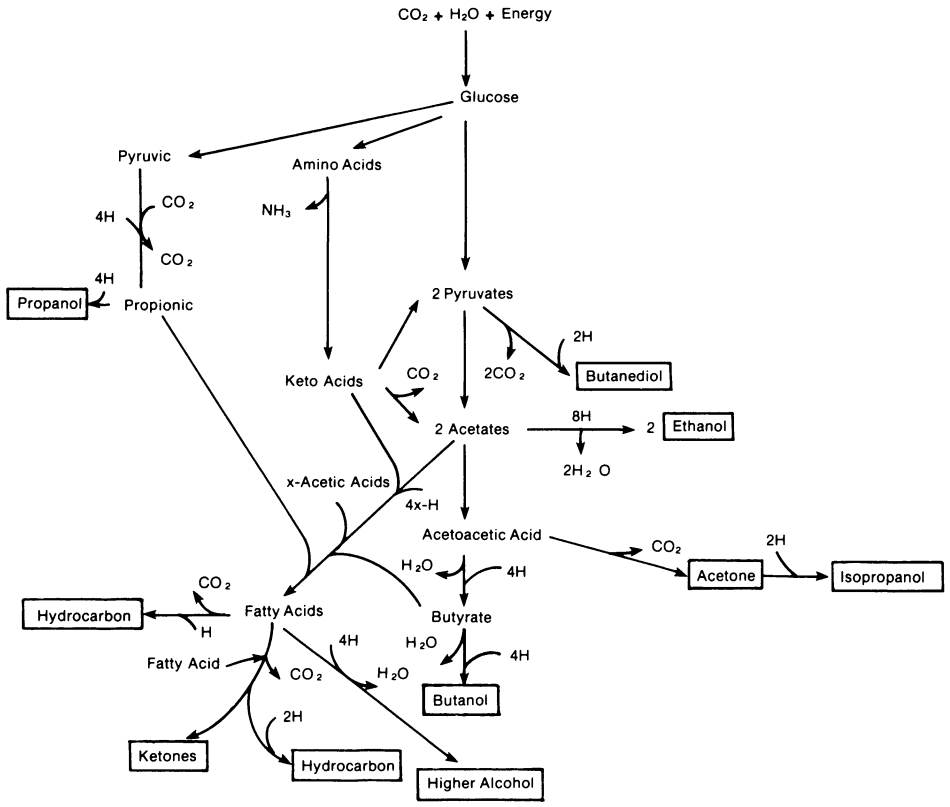
Multifaceted and diverse energy sources will replace our once massive accumulations of energy reserves. One of these energy sources will be biomass and its natural products; in fact, it will most certainly be one of the essential elements in the complex of the future energy structure.

Solar and chemical energy conversion, through biology as a practical energy conversion mechanism, has been extensively documented and reviewed; therefore, this discussion will be restricted to microbial fermentations with specific evaluations of the potentials for microorganisms to synthesize oily hydrocarbons as fermentation products. In biosynthesis, the acyclic hydrocarbons are referred to as fermentation products on the basis of the strict definition of fermentation as being those chemical energy yielding reactions that require organic components as electron acceptors. A generalized fermentation scheme is given in the figure. The scheme is purposely restrictive to emphasize products that are potential fuels. Each of the fermentation products represents a valuable energy form. The most efficient of these fermentation products, in terms of cost of production, cannot be fairly evaluated at this time because of the differences in cell cultivation requirements, product recovery, and most importantly, since many of these products via microbial fermentations are not yet sufficiently developed for commercial consideration. With increasing awareness of microorganisms which grow well or adapt to marginal, extreme or waste environments (taking into account the benefit value of these environments and the rising expenses of waste treatment) the distinct probability exists that the production costs in developing fermentation systems for fuel will become increasingly feasible and attractive. Although the compounds listed in the figure are acceptable fuels and are accessible through microbial processes, the obvious selection of a biochemical fuel for development cannot be determined at this time because not all systems have been adequately investigated. The competitive readiness of the different fermentation systems and the economics of producing each product as they become developed will automatically map out our course of action in years to come.



Fermentation end product of metabolism.
Hydrocarbons are natural metabolic products of many microorganisms. Intensive cultivation systems and regulatory metabolic mechanisms will have to be developed, however, before microorganisms can be used as hydrocarbon producers.

Historically the global energy source has been, for the most part, the hydrocarbon compounds. The reasons for this are clear; however, the massive availability of hydrocarbons is rapidly becoming reduced. Should the substitutes for these naturally occurring hydrocarbons be replaced with biologically produced hydrocarbons? Both hydrocarbons and oxygenated compounds (fig.), either of which could serve as an excellent source of liquid fuels, are synthesized by microorganisms. Some liquid fuels are given in the table so that the properties of hydrocarbons and oxygenated compounds may be compared. While the standard free energy of combustion and boiling points of the hydrocarbons and alcohols become more asymptotic with increasing chain length, the boiling point values of the corresponding hydrocarbons are always lower and therefore, are more easily volatilized. The properties of a liquid fuel that can be converted to a gaseous one provide greater handling, storage and combustion capabilities and thus explaining a wider utilization in today's market. The routes of formation of hydrocarbons and oxygenated compounds are mechanistically related, both being synthesized through fatty acid precursors (fig.); therefore, whatever one's preference, research on the development of either one is intertied with the other. The most important question that must be dealt with first, however, is whether microbial systems are capable of providing us with such compounds as hydrocarbons in sufficient quantities to be used as a commercial fuel directly, or to be used as a substrate for fuel production. As is typical of fermentation systems, only specific organisms have the potential to produce hydrocarbons; however, unlike the relatively established industrial microbial fermentation systems, applications of acyclic hydrocarbon syntheses are still in their infancy. The exact biosynthetic mechanism of hydrocarbon formation remains unknown, except that the routes of formation are obviously different in different microorganisms. Hydrocarbons are the end product of the reduction of organic compounds derived from decarboxylation, elongation-decarboxylation, or decarboxylation-condensation reactions of

Combustion properties of some liquid fuels

Name, formula (mol.wt)	Density (g/cm^3)	Boiling point (°C)	Heat of combustion kJ/mole	kJ/g
Oxygenated compounds				
Methanol CH_4 (32.04)	0.793	64.6	−727.3	−22.7
Ethanol C_2H_6O (46.1)	0.798	78.5	−1368.7	−29.7
Propanol (iso) C_3H_8O (60.1)	0.785	82.3	−2007.2	−33.4
Butanol (t) $C_4H_{10}O$ (74.1)	0.789	82.8	−2645.8	−33.7
Butanol (n) $C_4H_{10}O$ (74.1)	0.810	117.7	−2679.3	−36.2
Acetone C_3H_6O (58.1)	0.792	56.5	−1791.2	−30.8
Hydrocarbons				
Pentane C_5H_{12} (72.1)	0.626	36.2	−3511.9	−48.7
Hexane C_6H_{14} (86.2)	0.660	69.0	−4166.2	−48.3
Octane C_8H_{18} (114.2)	0.704	125.8	−5474.8	−47.9
Dodecane $C_{12}H_{26}$ (170.3)	0.766	214.5	−8092.4	−47.5

fatty acids[1–5]. 4 previously published reviews[6–9] outline what is known about these hydrocarbon producing microorganisms; therefore, only a brief overview of microbial hydrocarbons will be presented here. In a variety of marine and freshwater algae including red, green and brown, diatoms and phytoplankton, n-heneicosahexaene (C-21:6) hydrocarbons exist in amounts inversely correlated with the abundance of the long-chain highly unsaturated fatty acids (C-22:6)[3,10,11]. Structural assignments of the C-21:6 hydrocarbons from different sources are developing into a concise taxonomic picture[12,13]. This hydrocarbon is produced in quantities that make up more than 1% of the total dry weight of some species. In contrast, nonphotosynthetic diatoms and dinoflagellates contain traces of aliphatic hydrocarbons but no C-21:6. Other studies[14,15] found that n-pentadecane (C-15) predominates in brown algae, n-heptadecane (C-17) in red algae; that olefins predominate in red algae, more so than in brown or green algae; and that C-19 polyunsaturated olefins exist in red, brown, and green algae. The concentrations of these hydrocarbons are generally in minute to trace quantities. The occurrences of mixtures of monoenes, dienes and trienes with a C-15 to C-18 range in algae are relatively common, and, for the most part, exist in relatively small quantities[6]. Most hydrocarbon distribution patterns in blue-green bacteria represent a small percentage of the organic material and consist of predominantly C-17 components and the unique internal methyl branched 7,9-dimethyl hexadecane and a mixture of 7- and 8-methyl-heptadecane[6]. The lipid composition of the green algae *Dunaliella salina* comprised some 50% of the cellular organic material; more than 30% of the total lipids consisted of acyclic and cyclic hydrocarbons[16]. Carotenes accounted for 21% of the cell mass with another 3.5% being saturated and unsaturated C-17 straight chain hydrocarbons and internally branched ones identified as 6-methyl hexadecane and 4-methyl octadecane[16]. Further studies[21] demonstrated that temperature, light intensities and specific light spectra greatly influence the amount and type of lipids synthesized in this alga. In the chlorophyte, *Botryococcus braunii,* there exist 3 carbon distribution ranges of hydrocarbons, C-17, C-27 to C-31 and C-34 to C-36, depending on the algal growth phase. Approximately 0%, 17% and 86% of dry cellular weight is hydrocarbons of the green resting, green active and brown resting growth phases, respectively[6]. Initial reports on the hydrocarbon composition of this alga obviously contained contradictory data since different studies were performed on cells in different growth phases. The relationships of growth phase vs hydrocarbon synthesis suggest the possibility that inaccuracies may exist in all previous reports on the evaluation of hydrocarbon biosynthesis in microorganisms when culture age and environmental parameters were not considered.

Our understanding of the acyclic hydrocarbon composition of bacteria is no further advanced than that of algae. The quantities of hydrocarbons range from 35% of the 2% total lipids in some micrococci[6,17], 17.4% of the 7.4% of the cellular lipids of *Pseudomonas maltophilia*[18], 25% of the 5.9% cellular lipids of *Desulfovibrio*[19] to generally insignificant quantities in all bacteria[6] studied for hydrocarbon formation. The most thoroughly analyzed of the non-isoprenoid hydrocarbons are those of the taxonomic family Micrococcaceae. Their hydrocarbons are in the range from C-16 to C-32[6,7,17]. These hydrocarbons are a unique mixture of symmetrical and asymmetrical terminal branched monoene isomers that depict a specific chemotaxonomy profile[17]. The hydrocarbons are clearly a product of carboxyl end to carboxyl end condensations of 2 fatty acids with one of the fatty acids being decarboxylated prior to, or simultaneous with, the condensation[4,5]. The blocking of hydrocarbon synthesis in micrococci with Pb^{2+} resulted in long chain ketones that corresponded exactly to the chemical structure of hydrocarbons in carbon number as well as branching configuration[20,21]. All lines of evidence obtained support the proposal that the ketones are a metabolic end-product and not a precursor in the hydrocarbon synthesis[21]. These data demonstrated that the formation of hydrocarbons and ketones are important compounds in the regulation of the cellular fatty acid pool. This may well be the underlying basis for the formation of hydrocarbons in specific microorganisms and a fundamental key with which to unlock the mystery of the formation and regulation of hydrocarbons in microorganisms in general. In other bacteria, such as *Pseudomonas maltophilia*[18], there also exists a complex family of aliphatic hydrocarbon isomers in the range from C-22 to C-32 that are terminally methyl branched. These complex hydrocarbons have not been fully characterized, but it appears that the biosynthetic pathway follows the same general pathway as that described for micrococci. Numerous other studies have analyzed the hydrocarbon compositions of many more diverse bacteria with different nutritional properties and from different habitats[6,7]. Most of the quantities of hydrocarbon compositions found in bacteria were small ($< 1.0\%$) with the components being straight chains in the range from C-15 to C-31 with generally no predominance of odd over even numbered carbons. For example, the distribution of components of *Desulfovibrio* was in the range from C-15 to C-28 with the components intensities peaking at C-19, C-20 and C-21[19]. Many of the bacteria contain, in addition to the straight chain components, relatively trace amounts of isoprenoid hydrocarbons. For example, phototrophic bacteria contain pristane, (C-19), phytane (C-20), squalene (C-30) and/or carotenoid (C-40)[6,7]. There are diverse types of bacteria that have the isoprenoid hydrocarbons as the predominant ones and the non-

isoprenoid hydrocarbons as the minor ones[6,22–26]. The principal isoprenoid found most commonly is squalene (C-30); however, isoprenoids, hydroisoprenoids and isopranoids of different chain lengths exist in the range from C-15 to C-30 in a variety of bacteria, including phototrophs, halophiles, methanogens, thermophiles, and acidophiles[6,22–26]. These isoprenoids generally account for a maximum of around 1% of the cell mass. A most interesting relationship was observed between the isoprenoid content of some of these bacteria and the similar distribution of these components in ancient sediments and petroleum[23–25]. The contemporary bacteria which synthesize these isoprenoids exist in unusual environmental conditions similar to those thought to exist in various evolutionary stages of the archaen ecology. These findings were proposed to have major implications for biological and biogeochemical evolution[23–26]. These types of hydrocarbons produced by bacteria from unusual or extreme environments exist in the hydrophobic regions of the cells, namely the cytoplasmic membrane, and represent the type of compounds one might expect to enhance membrane stability and function of cells that must exist in such habitats. It has also been demonstrated that the isoprenoids function in some capacity as hydrogen storage compounds[26].

Fungal acyclic hydrocarbon biosynthesis is readily detectable in virtually all samples but it generally provides only small quantities of the total cellular constituents and metabolic products[6,8]. Hydrocarbons have been reported in yeast, fungal spores, and fungal mycelia. In general, the fungal hydrocarbons in the range from C-14 to C-37 are n-alkanes with no special distribution features that relate to a chemotaxonomic picture[6,8]. Hydrocarbon biosynthesis by several yeasts has been reported. Hydrocarbons accounting for more than 1% of the cell dry weight were reported for *Debaryomyces*[27]; but the hydrocarbon fractions in the range of C-16 to C-39 were not fully characterized. Yeast hydrocarbons are more typically alkanes and alkenes in the range from C-14 to C-34 with squalene often representing a significant portion of the less than 0.1% lipid of the dry cell weight composition.

This overview attests that hydrocarbons are a metabolic product of specific organisms, Hydrocarbon formation by cells is mechanistically no different from any other specific fermentation product produced by select organisms. Many years of intense research were spent to discover and develop strains which would maximize the production of existing commercial fermentation products. Similar efforts also will be required to develop a microbial hydrocarbon producing system. However, in spite of the potential for developing intensive cultivation programs and cell manipulation of such good neutral lipid producers as *Botyrococcus* and *Dunaliella*, we have not yet found the organism that is suited to develop into a hydrocarbon producer on a commercial scale. The reasons for the widespread excitement in this field lie in the existing but as yet undetermined genetic regulation and metabolic parameters that control hydrocarbon producing capacities.

The exploitation of photosynthetic microorganisms affords the only feasible approach to bioproduction of hydrocarbon oils. Photosynthetic microalgae: a) use sunlight as energy for biochemical synthesis from inorganic compounds; b) can be intensively cultivated in bodies of water generally considered unusable for domestic purposes; and c) accumulate trace metals and synthesize proteins, carbohydrates and vitamins thereby making excellent livestock feed or fertilizers. Particularly important is the fact that the cultivation of microalgae for the purpose of producing fermentation products is unlike other biomass programs in that all endproducts increase our biomass rather than decrease it. Such rewards make the search for the hydrocarbon producing algae a most exciting one.

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