5 Production of Microbial Biomass

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1 Historical Background

Human beings have used microorganisms since prehistoric times. The first utilization was accidental and then, after much trial and error, microorganisms were used empirically in the making of beverages, foods, textiles and antibiotics. It was not until 1830 that Cagniard de Latour and Kutzing and Schwann discovered that the growth of yeasts and other Protista caused the fermentation processes involved in the making of products such as wine.

In about 1850, extensive work by Pasteur resulted in considerable progress. Pasteur described bacteria and yeasts at a physiological level, introduced aseptic methods and the notion of minimum medium and defined nutrient and oxygen requirements.

Monod modeled bacterial growth in 1949. He launched the ideas of growth yield, specific growth rate and the concentration of growth-limiting substrates. The discovery of microorganisms and understanding of growth mechanisms made possible the development of culture methods. The theoretical approach to chemostat culture was performed by Monod and Novick and Szilard in 1950. Control of microbial cultures requires knowledge of the metabolic pathways involved, determination of the nutrient requirements of the microorganism and the physicochemical conditions required for optimum growth. Furthermore, development and changes in culture equipment thanks to progress in process engineering enable optimization of the production of microorganisms and their metabolites.

In addition to traditional agrofood processing (wine, beer, dairy products, bread, etc.), applications have been developed in the pharmaceutical sector (antibiotics, vitamins, etc.) and in the production of metabolites (enzymes and amino acids).

Many processes have been developed to produce microorganisms able to use organic material as a source of carbon and energy and to convert inorganic nitrogen into high-food-value proteins. These can be used in human foodstuffs or animal feed to replace traditional plant or animal sources.

Microorganisms such as algae, bacteria, yeasts and fungi have been considered as protein sources. Culture of microorganisms for their nutritional value started at the end of the First World War. The Germans developed yeast culture for use in animal and human diets. The term “fodder yeast” was coined (Braude, 1942; Weitzel and Winchel, 1932; Schulein, 1937). After the Second World War, production of fodder yeast using the pentoses in sulfite liquor was developed in the USA, and other processes were developed in the United Kingdom. Carbon substrates such as molasses, pulp of the coffee and seed pulp of the palmyra palm were used.

Interest in the production of microbial biomass as food and fodder intensified after the 1950s and reached a peak towards 1977. The term “Single Cell Protein (SCP)” was invented, and much information on these processes was published in a large number of journals (cf. References). The most important features resulting from research on SCP concerned the following topics:

- diversity of the substrates used and their catabolism (renewable substrate, fossil mass);
- breeding and genetic improvement of a great variety of microorganisms.

The various microorganisms used for biomass production and the various metabolic pathways involved in substrate catabolism are described here. Physiological aspects, growth parameters, energy and nutritional requirements and the influence of the physicochemical parameters are discussed. The different types of microbial culture and examples of processes are described.

2 Microorganisms

Four types of microorganisms are used to produce biomass: bacteria, yeasts, fungi and algae. The choice of a microorganism depends on numerous criteria, the most impor-
Tab. 1. Composition (g per 100 g DW) of Microorganisms of Interest in Biomass Production (LICHTFIELD, 1979)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Substrate</th>
<th>Nitrogen</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella sorokiniana</td>
<td>CO₂</td>
<td>9.6</td>
<td>60</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Spirulina maxima</td>
<td>CO₂</td>
<td>10</td>
<td>62</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Spirulina maxima</td>
<td>CO₂</td>
<td>8.5</td>
<td>53</td>
<td>4.8</td>
<td>28</td>
</tr>
<tr>
<td><strong>Bacteria and Actinomycetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulomonas sp.</td>
<td>Bagasse</td>
<td>14</td>
<td>87</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Methylomonas clara</td>
<td>Methanol</td>
<td>12–13</td>
<td>80–85</td>
<td>8–10</td>
<td></td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida lipolytica</td>
<td>n-Alkanes</td>
<td>10</td>
<td>65</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Candida utilis</td>
<td>Ethanol</td>
<td>8.3</td>
<td>52</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Kluyveromyces fragilis</td>
<td>Whey</td>
<td>9</td>
<td>54</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Molasses</td>
<td>8.4</td>
<td>53</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td><strong>Molds and Higher Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Molasses</td>
<td>7.7</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortella crassipes</td>
<td>Glucose</td>
<td>5</td>
<td>31</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>Sulfite waste liquor</td>
<td>8.8</td>
<td>55</td>
<td>1.3</td>
<td>25</td>
</tr>
</tbody>
</table>

The ideal microorganism should possess the following technological characteristics:

- high specific growth rate ($\mu$) and biomass yield ($Y_{x/s}$);
- high affinity for the substrate;
- low nutritional requirements, i.e., few indispensable growth factors;
- ability to use complex substrates;
- ability to develop high cell density;
- stability during multiplication;
- capacity for genetic modification;
- good tolerance to temperature and pH.

In addition, it should have a balanced protein and lipid composition. It must have a low nucleic acid content, good digestibility and be non-toxic (Tab. 1).

2.1 Yeasts

Yeast were the first microorganisms known, the best studied and generally best accepted by consumers. Yeasts are rarely toxic or pathogenic and can be used in human diets. Although their protein content rarely exceeds 60%, their concentration in essential amino acids such as lysine (6 to 9%), tryptophan and threonine is satisfactory. In contrast, they contain small amounts of the sulfur-containing amino acids methionine and cysteine. They are also rich in vitamins (B group), and their nucleic acid content ranges from 4 to 10%.

They are larger than bacteria, facilitating separation. They can be used in a raw state. However, their specific growth rate is relatively slow (generation time 2 to 5 hours).

2.2 Bacteria

The specific growth rate and biomass yield of bacteria are greater than those of the other categories of microorganisms. Total protein content may reach 80%. Their amino acid profile is balanced and their sulfur-containing amino acid and lysine concentrations are
high. In contrast, their nucleic acid content (10 to 16%) is greater than that of yeasts.

A limited number of bacterial species can be used in foodstuffs as many are pathogenic. In addition, separation is difficult because of their small size.

2.3 Fungi

The use of fungi as biomass is relatively new. They are more conventionally used for producing enzymes, organic acids and antibiotics. Their generation times (5 to 12 hours) are distinctly longer than those of yeasts and bacteria. This is generally an apparent generation time as they grow through elongation of mycelium; growth is not really exponential. Their protein content (50%) is often smaller than that of yeasts and bacteria, and they are deficient in sulfur-containing amino acids. There are also problems of wall digestibility. However, the nucleic acid content is low (3 to 5%).

The principal merits of fungi are their ability to use a large number of complex growth substances such as cellulose and starch and easy recovery by simple filtration, reducing production costs.

2.4 Algae

The potential merits of algae are related to their ability to multiply with CO₂ as the only carbon source. Some genera (Cyanophyta) can use atmospheric nitrogen. Algae production takes place in natural waterbodies (ponds, lakes and lagoons). Algae are traditionally a food complement for some populations in Mexico (Spirulina platensis) and Chad (Spirulina maxima). However, algae have a low sulfur-containing amino acid content. Their nucleic acid content is about 4 to 6%. They are easy to recover, but multiplication is very slow, and investment costs involved in artificial shallow ponds result in low process profitability.

2.5 Selection and Improvement of Strains

2.5.1 Selection of Microorganisms

The strategy for selecting microorganisms can be described schematically in several stages (Steele and Stowers, 1991). The first stage consists of:

- definition of the types of transformation sought (carbon substrate, type of microorganism) and definition and characterization of the product to be obtained (baker's yeast, wine yeast, food yeast);
- identification of the microorganisms capable of the transformations desired;
- selection of microorganisms in collections, the natural environment or a favorable environment (waste water, refuse, etc.);
- definition and development of screening.

The second stage consists of the study and comparison of several pre-selected strains. The main features investigated are the growth parameters (μ, Yx/s), the physicochemical parameters (pH, temperature, nutritional requirements) and the characteristics peculiar to each strain (cell composition, pathogenicity, toxicity, food value). All these studies can be performed in Erlenmeyer flasks or in a batch fermenter at laboratory scale.

After selection of the microorganisms displaying the best characteristics, the last stage consists of examining and determining the optimum growth parameters of the microorganism and choosing the most suitable culture procedure (batch, fed-batch, continuous, recycling, etc.). These studies should make it possible to extrapolate the laboratory-designed process for use at industrial scale.

2.5.2 Examples of the Selection and Improvement of Strains

The microorganisms involved in biomass production are mainly obtained by searching
for mutants occurring by natural selection, mutation or gene manipulation.

Heat-tolerant yeasts and bacteria have thus been selected for processes using methanol or the \( n \)-paraffins as the carbon source; the metabolisms of these substrates usually generate considerable heat.

A mutant of *Pichia pastoris* was selected with reduced biotin and thiamine requirements (IFP-Technip methanol process). The protein content has also been increased in molds such as *Trichoderma album* (INRA-Blachère process). A *Candida tropicalis* yeast was isolated for its high protein content and – mainly – for its high lysine content (IFP process on \( n \)-paraffins). The protein yields of a bacterial strain (*Methylophilus methylotrophus*) were increased after gene manipulation affecting its nitrogen metabolism (ICI process).

Strains with higher specific growth rates than normal have also been isolated. The obtaining of a mutant form of *Pichia pastoris* led to a 20 to 30% saving in production cost (IFP-Technip). The type of mutation sought is always closely related to the carbon substrate and the process used. The search for mutants with specific growth rate or even protein content features can generally be carried out by continuous multiplication and steady increase in the dilution rate. Screening techniques suitable for each case are used for the other characteristics.

### 2.6 Characteristics of Single-Cell Biomass

The food value of microorganisms is directly related to their protein and amino acid composition and their lipid, vitamin and nucleic acid contents. Various analyses must also be performed:

- overall analysis: water, lipid, protein, fiber and mineral contents;
- lipid analysis: proportions of fatty acids, sterols and phospholipids;
- analysis of nitrogen compounds: total nitrogen, amino acid profile, nucleic acid nitrogen, purine and pyrimidine base, quantification of RNA and DNA;
- analysis of minerals: major elements (Na, K, Mg, Ca, Cl) and trace elements (Mn, Zn, Cu, Fe, Co, Mo, As, Pb, Hg);
- analysis of carbohydrates;
- analysis of vitamins.

#### 2.6.1 Composition

The compositions of various microorganisms used for biomass production are shown in Tab. 1. The protein content is often defined as total Kjeldahl nitrogen \( \times 6.25 \). The real protein content is found using the sum of amino acids determined by analysis according to the recommendations of the Protein Advisory Group of the United Nations System (PAG) Guideline No 6. Comparison of the protein content of different microorganisms cultured on various carbon substrates with the protein content of egg and soy is shown in Tab. 2.

#### 2.6.2 Nutritional Value and Toxicological Status

Important aspects of the quality of the biomass produced are as follows:

- nutritional value of the product,
- safety of the product,
- production of protein concentrate free of nucleic acid and toxic substances.

Three parameters are used to establish the nutrient value of biomass: digestibility, biological value and protein efficiency ratio. Digestibility \( (D) \) is the percentage of total nitrogen consumed in relation to the nitrogen in the food ration: \( D = \left( \frac{I - F}{I} \right) \times 100 \). The total quantity of microbial protein ingested by animals is measured and the nitrogen content \( (I) \) is analyzed. Nitrogen contents in feces \( (F) \) and urine \( (U) \) are collected and measured. Digestibility of algae and bacteria is 83 to 88% and that of yeasts ranges from 88 to 96% (LITCHFIELD, 1979).

Biological value \( (BV) \) is the percentage of total nitrogen assimilated that is retained by the body, taking into account the simultaneous loss of endogenous nitrogen through urinary excretion: \( BV = \left( \frac{I - (F + U)}{I} \right) \).
Tab. 2. Essential Amino Acid Content of Microorganisms of Interest for Biomass Production (g per 16 g N) (LITCHFIELD, 1979; BOZE et al., 1992)

<table>
<thead>
<tr>
<th>Protein Source</th>
<th>Substrate</th>
<th>Cys</th>
<th>Ile</th>
<th>Leu</th>
<th>Lys</th>
<th>Met</th>
<th>Phe</th>
<th>Thr</th>
<th>Try</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO reference</td>
<td></td>
<td>2.0</td>
<td>4.2</td>
<td>4.2</td>
<td>2.2</td>
<td>2.8</td>
<td>2.8</td>
<td>1.0</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Soy bean meal</td>
<td></td>
<td>0.7</td>
<td>2.2</td>
<td>3.5</td>
<td>2.8</td>
<td>0.6</td>
<td>2.2</td>
<td>1.9</td>
<td>0.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Fish meal</td>
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<td>5.0</td>
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<td>3.0</td>
<td>0.9</td>
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<tr>
<td>Egg</td>
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<td>2.4</td>
<td>6.7</td>
<td>8.9</td>
<td>6.5</td>
<td>5.1</td>
<td>5.8</td>
<td>5.1</td>
<td>1.6</td>
<td>7.3</td>
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<tr>
<td>Algae</td>
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<td></td>
</tr>
<tr>
<td>Chlorella sorokiniana</td>
<td>CO₂</td>
<td>3.4</td>
<td>4.0</td>
<td>7.8</td>
<td>1.8</td>
<td>2.7</td>
<td>3.2</td>
<td>1.4</td>
<td>5.1</td>
<td></td>
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<tr>
<td>Spirulina maxima</td>
<td>CO₂</td>
<td>0.4</td>
<td>5.8</td>
<td>7.8</td>
<td>4.8</td>
<td>1.5</td>
<td>4.6</td>
<td>4.6</td>
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<tr>
<td>Cellulomonas alcaligenes</td>
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<td>7.4</td>
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<td>5.5</td>
<td>7.1</td>
<td></td>
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<td>Methylphilus methylotrophus</td>
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<td>4.3</td>
<td>6.8</td>
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<td>3.4</td>
<td>4.6</td>
<td>0.9</td>
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<tr>
<td>Thermomonospora fusca</td>
<td>Cellulose pulping</td>
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<td>3.2</td>
<td>6.1</td>
<td>3.6</td>
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<td>n-Alkanes</td>
<td>1.1</td>
<td>4.5</td>
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<td>7.0</td>
<td>1.8</td>
<td>4.4</td>
<td>4.9</td>
<td>1.4</td>
<td>5.4</td>
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<td>7.1</td>
<td>6.6</td>
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<td>Kluyveromyces fragilis</td>
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<td>6.1</td>
<td>6.9</td>
<td>1.9</td>
<td>2.8</td>
<td>5.8</td>
<td>1.4</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Molasses</td>
<td>1.6</td>
<td>5.5</td>
<td>7.9</td>
<td>8.2</td>
<td>2.5</td>
<td>4.5</td>
<td>4.8</td>
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<tr>
<td>Molds and Higher Fungi</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Molasses</td>
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<td>4.2</td>
<td>5.7</td>
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<td>1.9</td>
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<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>Sulfite waste liquor</td>
<td>1.1</td>
<td>4.3</td>
<td>6.9</td>
<td>6.4</td>
<td>1.5</td>
<td>3.7</td>
<td>4.6</td>
<td>1.2</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Variation is substantial: from 30 to 90% according to the microorganism (LITCHFIELD, 1979).

The protein efficiency ratio (PER) is the proportion of nitrogen retained by the animal in comparison with reference proteins, e.g., egg albumin.

Numerous nutritional and toxicological tests on animals are obligatory before any use in human or animal foodstuffs. These tests were published in a series of Guidelines (IUPAC, 1974) by the Protein Advisory Group (PAG) of FAO/WHO/UNICEF and IUPAC.

2.7 Microorganisms Used for Biomass Production

Numerous species of algae, bacteria, yeasts and fungi are used to produce biomass from the various carbon substrates available (GOLDBERG, 1985; ATKINSON and MAVITU-NA, 1983). A list (not exhaustive) is given in Tab. 3.

(\(I - F\)) \(100.\) Variation is substantial: from 30 to 90% according to the microorganism (LITCHFIELD, 1979).

In principle, the microorganisms mentioned here are not toxic, and it should be possible to use them all in animal feed and most in human food. However, they are not all officially accepted in all countries. It should also be noted that excessive use of antibiotics in the treatment of certain illnesses has resulted in the appearance of antibiotic-resistant strains (e.g., Candida tropicalis) of non-toxic species. Use of resistant strains should be avoided.

3 Carbon Substrates and Metabolic Pathways

3.1 Carbon Sources and Energy Sources

The variety of carbon sources available (CO₂, carbohydrates, hydrocarbons, lipids) makes it necessary to choose microorganisms with specific metabolic pathways. The various
Tab. 3. Microorganisms Used According to Carbon Source

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae</strong></td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td><em>Chlorella pyrenoidosa</em>, <em>C. regularis</em>, <em>C. sorokiniana</em>, <em>Oocystis polymorpha</em>, <em>Scenedesmus quadricaula</em>, <em>Spirulina maxima</em>, <em>Spirulina platensis</em>, <em>Dunaliella bardawil</em></td>
</tr>
<tr>
<td><strong>Bacteria and Actinomycetes</strong></td>
<td></td>
</tr>
<tr>
<td>n-Alkanes</td>
<td><em>Acinetobacter cerificans</em>, <em>Achromobacter delvacuatus</em>, <em>Mycobacterium phlei</em> sp., <em>Nocardia</em> sp., <em>Pseudomonas</em> sp.</td>
</tr>
<tr>
<td>Methane</td>
<td><em>Corynebacterium hydrocarbonoclastus</em>, <em>Nocardia paraffinica</em>, <em>Acinetobacter</em> sp., <em>Flavobacterium</em> sp., <em>Hyphomicrobium</em> sp., <em>Methylomonas methanica</em>, <em>Methylomomas capsulatus</em></td>
</tr>
<tr>
<td>Methanol</td>
<td><em>Methylomonas methylivorora</em>, <em>M. clara</em>, <em>M. methanolica</em>, <em>Flavobacterium</em> sp., <em>Methylphilus methylotrophus</em>, <em>Pseudomonas</em> sp., <em>Streptomyces</em> sp., <em>Xantomonas</em> sp.</td>
</tr>
<tr>
<td>Ethanol</td>
<td><em>Acinetobacter calcoaceticus</em></td>
</tr>
<tr>
<td>Cellulosic wastes</td>
<td><em>Thermomonospora fusca</em></td>
</tr>
<tr>
<td>Sulfite waste liquor</td>
<td><em>Pseudomonas denitrificans</em></td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
</tr>
<tr>
<td>n-Alkanes, n-paraffins</td>
<td><em>Candida lipolytica</em>, <em>C. tropicalis</em>, <em>C. guilliermondii</em>, <em>C. maltosa</em>, <em>C. paraffinica</em>, <em>C. oleophila</em>, <em>Yarrowia lipolytica</em></td>
</tr>
<tr>
<td>Methanol</td>
<td><em>Candida utilis</em>, <em>Hanseniaspora</em> sp., <em>Pichia pastoris</em>, <em>Hansenula</em> sp., <em>Kloeckera</em> sp.</td>
</tr>
<tr>
<td>Ethanol</td>
<td><em>Candida ethanomshophilum</em>, <em>C. utilis</em>, <em>C. kruzei</em></td>
</tr>
<tr>
<td>Whey</td>
<td><em>Kluyveromycos fragilis</em>, <em>Candida intermedia</em></td>
</tr>
<tr>
<td>Cane molasses</td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td>Starch</td>
<td><em>Schwannomyces alluavis</em>, <em>Lipomyces kononenkoe</em></td>
</tr>
<tr>
<td>Lipids</td>
<td><em>Candida rugosa</em>, <em>C. utilis</em>, <em>C. lipolytica</em>, <em>C. blankii</em>, <em>C. curvata</em>, <em>C. deformans</em>, <em>C. parapsilosis</em></td>
</tr>
<tr>
<td>Cellulose</td>
<td><em>Candida utilis</em></td>
</tr>
<tr>
<td>Sulfite waste liquor</td>
<td><em>Candida utilis</em>, <em>C. tropicalis</em></td>
</tr>
<tr>
<td><strong>Molds and Higher Fungi</strong></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td><em>Agaricus blazei</em>, <em>A. canpestris</em></td>
</tr>
<tr>
<td>Malt – molasses</td>
<td><em>Agaricus canpestris</em></td>
</tr>
<tr>
<td>Starch</td>
<td><em>Aspergillus niger</em>, <em>Fusarium graminearum</em></td>
</tr>
<tr>
<td>Sulfite waste liquors</td>
<td><em>Paeclomyces variotii</em></td>
</tr>
<tr>
<td>Cellulose</td>
<td><em>Chaetomium cellulolyticum</em>, <em>Trichoderma viride</em></td>
</tr>
<tr>
<td>Brewery waste</td>
<td><em>Calvatica gigantea</em></td>
</tr>
<tr>
<td>Carob bean extract</td>
<td><em>Aspergillus niger</em>, <em>Fusarium moniliforme</em></td>
</tr>
</tbody>
</table>

Microorganisms can be classified according to the carbon and energy sources that they are able to use (Atkinson and Mavituna, 1983) (Tab. 4).

The microorganisms used for biomass production are in the photolithotrophic and chemoorganotrophic categories. Special attention has been paid to the CO₂ metabolism of photolithotrophs and the hydrocarbon C1 compound, ethanol, glycerol, carbohydrate and lipid metabolism in chemoorganotrophs.

3.2 CO₂ Metabolism and Photosynthesis

CO₂ is the simplest carbon source for biomass production. It is present in the atmosphere at about 300 ppm. It is the most oxidized carbon source and cannot be used as an energy source. It can also be used in carbonate form. Energy is provided by light and converted into chemical energy by the photosynthesis mechanism.