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## Optimal growth rate conditions of microorganisms

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### ABSTRACT

The aim of review was to evaluate optimal conditions for microorganisms growth from different taxa, including yeast, bacteria and algae. Maximal efficiency of yeast *Saccharomyces cerevisiae* propagation occurs in aerated and constant low concentration of sugar medium. Optimal conditions for effective fermentation require high concentration of sugar which is converted into ethanol, however the produced alcohol cause stress and too high concentration of alcohol limit yeast growth. Bacteria such as *Escherichia coli* are also found to produce FAME, a particular product where efficiency of production strictly depends on FFA concentration. Algae *Chlorella vulgaris* accumulates lipids under nitrogen limit, but inhibited cell growth and division results. Optimal conditions of microorganisms growth depends on expected products and sources which are necessary to initiate particular metabolic pathway, required for production of specific product.

**Keywords:** bacteria *Escherichia coli*; yeast *Saccharomyces cerevisiae*; alga *Chlorella vulgaris*

## 1. INTRODUCTION

Microorganisms are a group of single-celled, microscopic organisms that are classified in the different taxonomic units. This concept includes inter alia kingdom *Bacteria*, *Archaea*, *Protista*, *Chromista* and certain representatives of the kingdom *Plantae* (*Chlorophyta*), representatives of *Fungi* (*Saccharomycetes*). Microbes live in almost any environment; there are known species that are extremely thermophilic and species which prefer extremely cold conditions [17]. Microbes can be absolute aerobes or anaerobes organisms or can tolerate both environments by changing the metabolic strategy depending on the presence or absence of oxygen. The diversity of microorganisms is tremendous. Among them are certain species widely used by man as an important part of industrial biotechnology [5,17].

The aim of study was to evaluate optimal conditions for microorganism growth from different groups, including yeast, bacteria and the artificial group of algae.

## 2. BACTERIA ESCHERICHIA COLI

### 2. 1. *Escherichia coli* main metabolism

*Escherichia coli* (*E. coli*) is a facultative anaerobic single-cell organism capable of using wide spectrum of organic carbon compounds. As a facultative anaerobic *E. coli* uses different strategies for energy production, such as fermentation or respiration. In most studies that describe the use of *E. coli* for applied processes, sugars are used as carbon and electron source [2]. Import of these sugars are driven by different mechanisms. For example glucose is mainly imported and phosphorylated to glucose-6-phosphate by phosphotransferase system [1]. After transportation and phosphorylation of glucose the glycolysis pathway can begin which enables to process phosphorylated sugar into two molecules of pyruvate, two molecules of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). When oxygen is available in the surrounding environment of *E. coli*, pyruvate is converted to acetyl coenzyme A (acetyl-CoA) and carbon dioxide by pyruvate dehydrogenase complex. Under oxidative conditions acetyl-CoA is further transformed in the citric acid circle. *E. coli* can respire in anoxic conditions using different electron acceptors as an oxygen replacement [29]. In the oxygen absence the citric acid cycle is downregulated causing incomplete sugar oxidation. In that case acetate is formed as the main product. When fermentative conditions occurs different amounts of ethanol, formate, acetate and lactate are produced maintaining redox balance in *E. coli* organism [7].

### 2. 2. *E. coli* growth conditions

The natural habitat of *E. coli* is the intestinal tracts of humans and vertebrate animals. However, water environments are recognized as a secondary habitat for certain *E. coli* strains [27]. Therefore there are some factors other than available carbon source which must be met for those strains so that they can survive in other habitats which are not natural for them. There are many models that predict growth conditions of *E. coli*. Some models describes the effect of water activity and temperature on *E. coli* growth rate, others add more variables such as pH [16,19]. Model based on growth rate data containing upper and lower limiting temperature, upper and lower limiting pH, minimum inhibitory concentrations of dissociated and un-dissociated lactic acid and lower limiting water activity described range of variables

necessary in *E. coli* growth. The variables where function of temperature (7.63-47.43 °C), water activity (0.951-0.999, adjusted with NaCl), pH (4.02-8.28) and lactic acid concentration (0-500 mM) [28]. With these combined range of temperature, water activity, pH and lactic acid concentration *E. coli* cells can survive, but when their environment has a suitable carbon source they can grow.

### 2. 3. *E. coli* growth by product

*E. coli* strains which are engineered by a variety of methods to produce specific products need to be grown in appropriate conditions or they will not make the desired substance. Such strains have additional plasmids containing genes from another species. This process was used in an experiment conducted by Nawabi et al. at [24] to produce biodiesel in *E. coli* cells, which is defined as fatty acid mono-alkyl esters and fatty acid methyl esters (FAMEs), the most common form. In these strains there was an addition of other necessary factors, such as Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) which induce heterologous gene transcription, because without it there would not be any proteins required to perform reactions leading to FAMEs. Another necessary factors to produce FAMEs where free fatty acids (FFA) or 3-OH-FFA [24]. In an experiment conducted by Martinez et al. in 2007 on two *E. coli* recombinants concluded that it is possible to reduce by 65% salts in medium without decreasing yield of productivity lactate from glucose or ethanol from xylose. Another interesting experiment that included altered *E. coli* strain was conducted by Schen et al., [6] entailing that *E. coli* strain had modified its fermentation pathways, by eliminating the reactions to use acetyl-CoA and oxidize NADH. These metabolic modifications allowed to increase flux through the n-butanol producing pathways. All of those previous experiments allow the conclusion that optimal growth conditions for *E. coli* depends mostly on the sole purpose which they are being used for. In example, if the intent is to produce FAME and the other general growth conditions such as temperature, pH and carbon source are met the only restrictive factor will be the amount of FFA.

### 3. YEAST

Yeast are organisms used in many industrial fields to produce food, cosmetic ingredients, medicines and biofuels. One of the most important characteristics of yeast which exploited in the food production industry is fermentation, the process where sugar is converted to ethanol. Yeast fermentation metabolism differs among species between non-, facultative- or obligate fermentative. *Saccharomyces cerevisiae* yeast are able to change from flexible oxygen respiration to fermentation, and the metabolic strategy depends on environmental resources [13,21].

The phenomenon of fermentation in oxygen presence is called Crabtree effect, but the evolutionary explanation calls it an ecologic strategy "Make Accumulate Consume" [14]. Yeast in some cases such a high content of sugar use all sources to make ethanol, which is an inexpensive product, provides ATP faster than respiration, furthermore ethanol limit growth of other competitive microorganisms. Optimal conditions for rapid yeast fermentation is high content of sugar, which depending on the strain can range between 5-20% of sugar. There are also some extremely effective yeast strains used in high gravity beers or, wine or distiller's production, where yeast ferment more than 25% of sugar, however those

conditions are quite severe and can cause stress for yeast. There are many sources of stresses as high begin density, high ethanol concentration (which is produced during fermentation process) and also amino acids and nitrogen deficiency appeared especially in stationary phase [9,15]. Direct consequences of stress-full conditions are longer growth process as well as in more incidental products [9,15].

Nitrogen deficiency affects efficiency of fermentation, resulting in higher alcohols and aromatic compounds production such as esters, fatty acids and isoacids as well. Nitrogen source is necessary for rapid and effective fermentation and also for yeast propagation. Yeast are able to reproduce sexually (sexual spores mating asexually (budding)). The process of population growth is based on budding where from mother cell 40-50 daughter cells are produced. The optimal conditions for population growth to produce maximal yeast yield is with strong aeration and permanent supplementation of nitrogen and also sugar. Moreover biomass production increased the fastest in permanent flow and constant portions of sugars in particular amounts and kept on stable low level, to avoid metabolic switch on fermentation and Crabtree effect. Supporting yeast in oxygen and low stable concentration of sugar directs yeast metabolism more on propagation than on fermentation. Medium is usually aerated by sterile air, not just oxygen (more air there means more efficient mixing, just oxygen might cause stress). In bioreactors where yeast are propagated are also used stirrers or mixers, speed and flow are adjusted to species and strain. Optimal growth conditions for yeast strictly depend on strain and also on metabolic pathway which is expected to be used.

#### 4. ALGAE

Algae is a polyphyletic group of simple, autotrophic organisms occurring in the water and damp environments. This ecological group occurred as a single cells, multicellular clusters (colonies), however their construction is not based on the tissue organization. *Chlorella sp.* (*Eukaryota*, *Viridiplantae*, *Trebouxiophyceae*) is one of the most commonly cultivated algae on the whole world (by at least a few dozen of companies). It is also one of the most examined microorganism [25] commonly used for dietary supplements, as a component of cosmetics [3,25] or as a raw material to production of biofuels. The annual production of biomass *Chlorella* reaches up to several thousand tons [3,23,25]. *Chlorella* is cultivated under photoautotrophic conditions, [10,12] or heterotrophically – in fermentors [10]. The biggest closed harvesting system (photobioreactor) using commercial, produces annually about 100 tonnes of *Chlorella* biomass [22].

Optimal conditions for algae growth depends on the objective, for instance to improve protein concentration (with required amino-acids) or increase lipid fraction of cell. Algae growth is based on light, however to increase growth rate there are necessary other factors as a nutrients supplementation, carbon dioxide sources, oxygen. Efficiency of algae growth depends strictly on pH (species preferences are many, there are acidophils and alkalophilic, however most of algae prefer neutral pH) and temperature of incubation. In order to modify the composition of these microorganisms, genetic manipulation or change in culture medium can be utilized. Restricting compounds, such as nitrogen and phosphorus causes redirection of the metabolic pathway to fatty acids synthesis. Research and test are also conducted to obtain organisms without enzymes that determine of starch accumulation (instead of lipids).

The restriction of access to the building compounds, such as nitrogen and phosphorus, causes redirecting the metabolic pathway in the synthesis of fatty acids. *Chlorella* is able to accumulate under stress large amounts of lipids [3] or synthesized starch instead, for instance by applying sulphur limitation, increased starch concentration of *Chlorella* up to 50% [11]. Inhibition of cell division occurs on assimilation and conversion of excess carbon to fatty acids and lipids [3,11]. *Chlorella vulgaris* accumulates lipid under nitrogen limitation and consequently decreases efficiency of biomass production. Improved lipid productivity may be compromised, despite higher lipid content. The highest volumetric lipid concentration and lipid productivity occurs respectively at nitrate concentrations of 305 and 241 mg L<sup>-1</sup>. There is a strong correlation between the nitrogen content of the cells and the pigment, lipid and protein content, as well as biomass and lipid productivity. Nitrogen limitation improves the lipid profile for biodiesel production [20]. Cell division discontinues after depletion of nitrates from the growth medium. The growth of non-dividing cells leads to the rapid accumulation of lipids. However, replenishment of nitrogen in to the medium, causes that lipid concentration decreased rapidly. The large lipid body becomes fragmented into smaller ones within 24 hours. This mobilization of the cellular lipid store occurred independently of light [26].

## 5. CONCLUSIONS

Microorganisms optimal growth conditions strictly depend on initiated metabolic pathway and purpose of growth. Yeast *Saccharomyces cerevisiae* optimal conditions are different for growth on biomass (yield - propagation) and fermentation. Maximal efficiency of propagation is possible where is used low, constant concentrations of sugars and permanent aeration. The opposite conditions as high concentration of sugar and low or even non aeration cause that yeast metabolism is bent on fermentation and ethanol production. Bacteria as *Escherichia coli* are also directed to produce some particular product as FAME, where efficiency of production strictly depends on FFA concentration. *Chlorella vulgaris* accumulates lipid under nitrogen limitation, what is used to initiate maximal lipid accumulation in cells, and optimal conditions to improved fatty acids concentration is based on nitrogen limitation. The conclusion of review is that microorganism are comparable to micro-size factory where are produced many of products as proteins, enzymes, lipids and substance like ethanol. Optimal conditions of growth depends on expected products and sources which are necessary to initiate particular metabolic pathway, required for production of specific product.

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