Media for Industrial Fermentations

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Introduction

The production of foods and beverages from fermentable carbon sources by microorganisms represents the oldest and most economically significant of all biotechnologies. A wide array of plant- and animal-based complex media for the industrial cultivation of bacteria, fungi, and yeasts are employed in the food industry (Table 1).

The composition of a fermentation medium in terms of nutrient bioavailability, and the absence of potentially toxic or inhibitory constituents, are crucially important for the metabolism and growth of food microorganisms. The cost of the medium is also important – raw materials account for a significant proportion (generally more than 50%) of the overall costs of production of a fermented food. Historically, the choice of media for large-scale fermentations has been based on price and availability rather than on microbial physiology.

Microorganisms require appropriate supplies of major, minor, and trace nutrients and water to metabolize and grow. The sources of these nutrients in media commonly employed in industrial food fermentations are described in the following sections.

Sources of Utilizable Carbon

All food microorganisms are chemoorganotrophs, with the exception of some photosynthetic microalgae – that is, they

Table 1 Selected fermentation media for food microorganisms

Media	*Microorganisms*	*Products*
Barley malt wort	Yeasts (*Saccharomyces* spp.)	Ale and lager beer, Scotch malt whisky, spent yeast (for yeast extracts), spent grains for animal feed
Cereal wort based on barley malt plus unmalted cereals (e.g., rye, wheat, maize, sorghum)	Yeasts (*Saccharomyces* spp.)	Some beers, Scotch grain whisky (yielding blended Scotch on mixing with malt whisky), bourbon whiskey, neutral spirits (e.g., gin, vodka, liqueurs), spent grains for animal feed
Ethanol	*Acetobacter* spp.	Vinegar (e.g., from beer, wine, cider)
Rice hydrolysate	*Aspergillus oryzae*, yeasts	Pachwai, saké, sochu, arrack, binuburan
Extracts of potatoes, artichokes, *Agave* spp., sweet potatoes	Yeasts	Aquavit, vodka, pulque, tequila, awamori
Sugarcane and sugar beet molasses	Yeasts, fungi, bacteria	Yeast biomass for baking, brewing, wine-making and distilling; yeast extracts/enzymes; rum, citric acid, glutamic acid
Wine must, fruit juices, honey	Yeasts, lactic acid bacteria	Wine, cognac, armagnac, brandy, grappa, kirsch, slivovitz, cider, perry, mead
Milk, cheese whey	Lactic acid bacteria, yeasts, fungi	Bacterial and fungal starter cultures for dairy produce (cheese, yogurts, buttermilk, sour cream, acidophilus milk, koumiss, taette, kefir); probiotics; lactic acid; ethanol (for potable spirits, cream liqueurs); spent yeast/food yeast
Starch hydrolysates, glucose syrups	Fungi, yeasts, bacteria	Mycoprotein; fermented beverages; microbial proteases, lipases, carbohydrases, organic acids
Water, CO_2, sunlight	*Chlorella*, *Scenedesmus*, *Spirulina*	Food sources; omega-3 fatty acids; protein/vitamin supplements
Solid substrate media		
Soya, wheat	*Aspergillus oryzae*, yeasts, lactic acid bacteria	Soy sauce (shoyu), tofu, tempeh, miso
Wheat flour	Yeasts, lactic acid bacteria	Bread, sourdough breads, rye breads, pumpernickel
Peanut press cake	*Neurospora sitophila*	Ontijom
Meat, fish	Lactic acid bacteria, fungi	Sausages (*Pediococcus cerevisiae*), fish sauces (halophilic *Bacillus* spp.), cured hams (*Aspergillus* and *Penicillium* spp.)
Plants, vegetables	Lactic acid and other bacteria	Sauerkraut (cabbage), pickles (e.g., cucumber), olives, tea, cocoa, coffee (pectinolytic *Bacillus* and *Erwinia* spp.)
Straw, manure, sawdust	*Pleurotus* spp.	Oyster mushroom
	Agaricus bisporus	Button mushroom
	Volvariella volvacea	Chinese (paddy-straw) mushroom
Oak wood	*Lentinula edodes*	Shiitake mushroom
Milk, curd	*Penicillium* spp.	Mold-ripened cheeses
	Propionibacterium spp.	Swiss-type cheeses
Wheat bran	Fungi (e.g., *Aspergillus niger*)	Food-processing enzymes
Tea leaves	*Acetobacter xylinum*, *Schizosaccharomyces pombe*	Teekwass

obtain their carbon and energy by metabolizing organic substrates. These include carbon biopolymers (e.g., starch, pectin), hexose and pentose monosaccharides (e.g., glucose, xylose), disaccharides (e.g., sucrose, maltose), trisaccharides (e.g., maltotriose), oligosaccharides (e.g., maltodextrins), alcohols (e.g., ethanol), polyols (e.g., glycerol), organic acids (e.g., lactic acid, acetic acid), fatty acids, amino acids, peptides, and polypeptides. All biosynthetically produced organic compounds of plant or animal origin have the potential to serve as substrates for microbial fermentation, but the capability of microorganisms of using particular carbon sources varies between genera and species. Sugars represent the main fermentable carbon sources for food microorganisms. The main catabolic and anabolic fates of sugars in microbial metabolism are outlined in Figure 1.

A small proportion of the carbon assimilated by chemoorganotrophic bacteria, fungi, and yeasts may be in the form of CO_2. This is 'fixed' using anaplerotic enzymes, such as phosphoenolpyruvate carboxykinase and pyruvate carboxylase.

Although glucose commonly is used as the sole carbon and energy source for the growth of food microorganisms in the laboratory, it generally is not freely available in industrial fermentation media. In these media, the more common carbon sources are maltose, sucrose, fructose, xylose, and lactose. Indeed, glucose frequently exhibits a repressive effect on the assimilation of other sugars by microorganisms. This is known as catabolite repression and is experienced, for example, by the yeast *Saccharomyces cerevisiae* during fermentation of complex sugar mixtures found in malt wort and cereal dough. Molasses, corn steep liquor, and sulfite waste liquor are complex carbon sources that also supply nitrogenous and mineral nutrients (Table 2).

Molasses, derived from the refining of sugar-rich plants to crystalline sucrose, is a globally employed fermentation substrate. Its composition varies with the specific production process and the geographic location. Different names are applied to molasses, depending on the mode of sugar production from which it was recovered. Thus, blackstrap molasses is the residual liquor following the crystallization of sucrose from sugarcane; beet molasses is generated similarly from sugar beet; refinery molasses differs from blackstrap molasses only in that it is the residual mother liquor that accumulates in the refining of crude sucrose by recrystallization; high-test molasses contains much of the original sugar of cane juice, which has been partially hydrolyzed (inverted) to glucose and fructose; and hydrol is molasses resulting from the production of crystalline glucose from corn starch. Table 3 provides more quantitative information on the composition of cane and beet molasses.

Corn steep liquor is the water extract (concentrated to 50% solids) that results from the steeping of maize during the production of corn starch, gluten, and other corn products. It contains high levels of lactic acid, resulting from the growth of lactic acid bacteria and fungi. Corn steep liquor is thus a natural product of fermentation. Sulfite waste liquor is the spent liquor from the paper-pulping industry, and it remains after wood is digested to cellulose pulp by calcium bisulfite under heat and pressure. Sulfite waste liquor contains 10% solids, of which 20% is composed of sugars (hexoses and pentoses). Sulfite waste liquor cannot be fermented directly – the free SO_2 or sulfurous acid must first be removed by steam stripping or precipitation with lime.

Molasses, corn steep liquor, and sulfite waste liquor generally represent complete nutritional sources for the growth of microorganisms, but certain components may be limiting, unavailable, inhibitory, or toxic. For example, molasses for the propagation of bakers' yeast or potable alcohol fermentations needs to be supplemented with assimilable nitrogen and phosphorus sources (e.g., in the form of diammonium hydrogen phosphate).

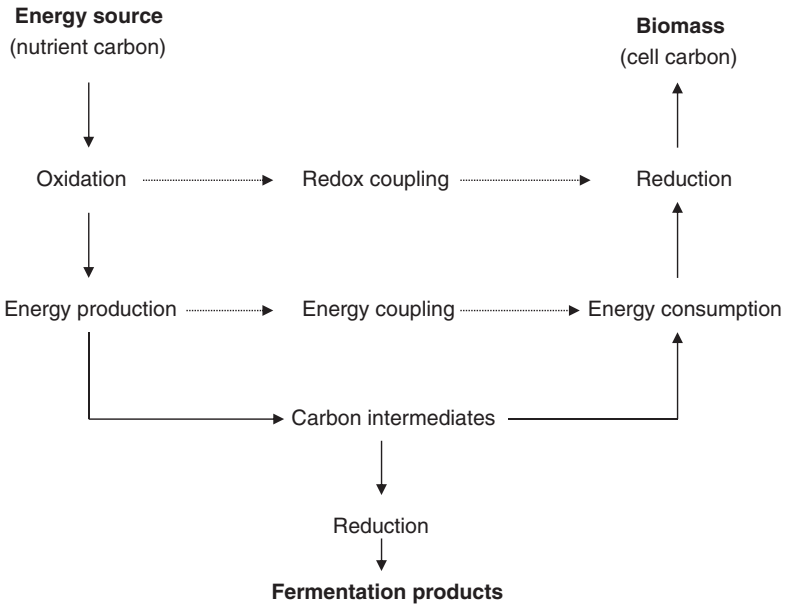

Figure 1 Overview of microbial carbon metabolism.

Table 2 Principal constituents of selected media used in food fermentations

	Molasses	*Beer wort*	*Wine must*	*Cheese whey*	*Corn steep liquor*
Carbon source	Sucrose Fructose Glucose Raffinose	Maltose Glucose Maltotriose Maltodextrins Sucrose Fructose	Glucose Fructose	Lactose	Glucose Other residual sugars
Nitrogen source	Protein Other nitrogenous compounds	Amino acids Ammonium ions Amino nitrogen compounds	Amino acids Amino nitrogen compounds	Amino and urea nitrogen compounds Globulin Albumin	Amino acids Peptides
Minerals	Phosphorus Potassium Magnesium Sulfur	Phosphorus Potassium Magnesium Sulfur	Phosphorus Potassium Magnesium Sulfur (sulfite often present)	Phosphorus Potassium Magnesium Sulfur	Phosphorus Potassium Magnesium Sulfur
Trace elements	Range present, but manganese (Mn^{++}) may be limiting	Range present, but Zinc (Zn^{++}) may be limiting	Range present	Iron Zinc Manganese Calcium Copper	Range present
Vitamins	Range present, but biotin may be deficient in beet molasses	Range present, but biotin may occasionally be deficient	Range present	Range present	Biotin Pyridoxine Thiamine
Other components	Unfermentable sugars Organic acids Waxes Pigments Silica Pesticide residues Caramelized compounds Betaine	Maltodextrins not fermented by yeasts Pyrazines Hop compounds	Pentose sugars not fermented by yeasts Tartaric and malic acids Decanoic and octanoic acids	Lipids NaCl lactic and citric acids	High levels of lactic acid present Fat Fiber

Sources of Utilizable Nitrogen

The nitrogen in microbial growth media serves an anabolic role in the biosynthesis of structural proteins and functional enzymes and nucleic acids. Some nitrogen sources, notably amino acids, may be catabolized immediately on entry into the cell, and the products may be important in determining the flavor of certain foods (e.g., higher alcohols and diacetyl in fermented beverages). Food microorganisms are non-diazotrophic (i.e., cannot fix atmospheric N_2), and therefore require a supply of either organic or inorganic nitrogen sources (Table 4).

Simple inorganic nitrogen sources such as gaseous ammonia or ammonium salts are utilized widely. Ammonium sulfate and diammonium hydrogen phosphate also are useful as sources of assimilable sulfur and phosphorus, respectively. Nitrate and urea may be employed as nitrogen sources: The former may be reduced to ammonia by many bacteria, fungi and yeasts, using the assimilatory enzyme nitrate reductase; urea may be used as an inexpensive nitrogen source in certain industrial fermentation media (e.g., molasses). Urea, however, is not recommended for the production of potable spirit beverages by yeast fermentation, because of the possible formation of carcinogenic ethylcarbamate during the distillation process.

Complex, organic forms of nitrogen are found in various types of hydrolyzed plant protein material (Table 5). For example, corn steep liquor, casein hydrolysate, soybean meal, barley malt, and yeast extract provide mixtures of peptides and amino acids that invariably support higher rates of growth and fermentation than those achieved using inorganic nitrogen sources. Peptones are protein hydrolysates derived from meat, casein, gelatin, keratin, peanuts, soybean meal, cottonseeds, and sunflower seeds, but they are relatively expensive sources of nitrogen for industrial applications. The individual amino acids present in complex mixtures may be assimilated sequentially by food microorganisms, but the presence of ammonium ions may inhibit amino acid uptake (due to nitrogen catabolite repression). In the case of some microbes, the provision of amino acids in the form of peptides may result in better growth than the provision of the same amino acids in free form.

Ammonia may be assimilated by either the glutamate dehydrogenase pathway or the glutamine synthetase–glutamate synthase pathway. In the former pathway, the reductive amination of α-ketoglutarate forms L-glutamate, while in the latter pathway, L-glutamate is aminated by glutamine synthetase to form L-glutamine. One of the amide groups of L-glutamine then is transferred to α-ketoglutarate by glutamate synthase, yielding two molecules of L-glutamate. The precise

Table 3 Composition of cane and beet molasses

Main constituent	*Components*	*Typical composition (% weight except for vitamins, mg kg^{-1})*	
		Cane molasses	*Beet molasses*
pH		5–6	7–9
Sugars	Total sugars	50–65% w/w	49–58% w/w
	Sucrose	30–40	47–55
	Invert sugar	10–25	0.2–2.0
	Nonfermentable sugars	3–5	1.0
Other carbon compounds	Gums, starch pentosans, hexitols, organic acids, waxes	10–15	10–20
Nitrogenous compounds	Crude protein, amino acids and other nitrogenous compounds	3.0	8–12
Minerals	Phosphorus	0.10	0.02
	Potassium	3.0	5.0
	Sulfur	0.55	0.33
	Magnesium	0.35	0.12
	Calcium	0.74	0.23
	Sodium	0.25	0.5
	Ash	9.0	5.0
Vitamins	Thiamine	1.8 mg kg^{-1}	1.3 mg kg^{-1}
	Riboflavin	2.5	0.40
	Pyridoxine	5	5
	Nicotinic acid	200	50
	Pantothenic acid	60	100
	Folic acid	0.04	0.20
	Biotin	1.2	0.05
	Choline	750	500
	Inositol	6000	8000

Figures quoted are representative of typical molasses – composition will vary depending on country of origin, method of production, etc.

Table 4 Commonly employed nitrogen sources in food fermentation media

Organic N sources	*Inorganic N sources*
Corn steep liquor	$(NH_4)_2SO_4$
Casein hydrolysate	NH_4Cl
Soybean meal	NH_3
Yeast extract	$(NH_4)_2HPO_4$
Barley malt	$(NH_4)_2PO_4$
Dried distillers grains with solubles (DDGS)	NH_4NO_3
Pharmamedia (cottonseed flour)	NH_4OH
Corn gluten meal	Urea
Linseed meal	
Rice and wheat meal	

pathway adopted depends on the microbial species, the concentrations of available ammonia, and the intracellular amino acid pools.

Sources of Inorganic Ions

Around 8% of the dry weight of microbial cells includes inorganic ions. The 'bulk' of macronutrients are required in millimolar concentrations and are nitrogen, phosphorus, sulfur, potassium, and magnesium. Micronutrients (or trace elements) are required in micromolar or less concentrations and play specific metabolic roles. They include sodium, calcium, chlorine, iron, cobalt, zinc, molybdenum, copper, manganese, nickel, and selenium. Several metal ions may be toxic to microorganisms at μmol l^{-1}concentrations, for example, silver, arsenic, barium, cesium, cadmium, mercury, lithium, and lead. Table 6 summarizes the major requirements of microorganisms in terms of inorganic ions.

Media for fermentations usually contain around 70–90% water, which acts as the solvent for the nutrients contained in the media and also supplies trace metals. The ionic content of the water used is influential in determining product quality, for example, the flavor of beer. Most of the complex fermentation media used in industry, and the water used to dilute such media, normally contain adequate levels of inorganic ions for microbial growth. Supplementation with additional minerals, however, occasionally may be necessary due to certain metals being present in concentrations that are suboptimal for efficient fermentation. Also, the bioavailability of metal ions may be compromised as a result of sterilization, precipitation, chelation, or binding to inert surfaces. The separate sterilization of metal ion supplements then may be necessary to counteract such losses (see section on Media Sterilization). Some metals may interact antagonistically – for example, the inhibition of essential magnesium-dependent cellular functions by calcium. In contrast, however, some metals may act synergistically, for example, magnesium and cobalt in fermentations involving

Table 5 Nitrogenous components of selected fermentation media

Medium	Dry matter (%)	Total protein (%)	Individual amino acids (%)												
			Arg	Cys	Gly	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Tyr	Val
Corn steep liquor	50	24	0.4	0.5	1.1	0.3	0.9	0.1	0.2	0.5	0.3	–	–	0.1	0.5
Dried distillers solubles	92	26	1.0	0.6	1.1	0.7	1.6	2.1	0.9	0.6	1.5	1.0	1.0	0.7	1.5
Pharmamedia (cottonseed flour)	99	59	12.3	1.5	3.8	3.0	3.3	6.1	4.5	1.5	5.9	3.3	0.95	3.4	4.6
Soybean meal	90	45	3.2	0.7	2.9	1.1	2.3	3.4	3.0	0.7	2.1	1.9	0.6	1.7	2.4
Wheat flour	90	13	0.8	0.2	–	0.3	0.6	1.0	0.5	0.2	0.7	0.4	0.2	0.5	0.6
Whey powder	95	12	0.4	0.4	0.7	0.2	0.7	1.2	1.0	0.4	0.5	0.6	0.2	0.5	0.6
Linseed meal	92	36	2.5	0.6	0.2	0.5	1.3	2.1	1.0	0.8	1.8	1.4	0.7	1.7	1.8
Brewers' yeast	95	43	2.2	0.6	3.4	1.3	2.7	3.3	3.4	1.0	1.8	2.5	0.8	1.9	2.4
Yeast autolysate	70	55	2.1	0.3	1.6	0.9	2.0	2.9	3.2	0.5	1.6	1.9	0.8	–	2.3
Casein hydrolysate	97	15	0.5	0.07	0.9	0.5	1.1	2.9	1.3	1.1	0.9	1.3	0.01	0.5	1.7

Table 6 Inorganic ion requirements of microorganisms

Element	Common source	Typical concentrations needed for growth	Cellular functions
Macronutrients			
Phosphorus and nitrogen	$(NH_4)_2HPO_4$	10 mmol l^{-1}	Energy transduction; nucleic acid and membrane structure
Potassium	KCl	5 mmol l^{-1}	Ionic balance; enzyme activity
Magnesium and sulfur	$MgSO_4 \cdot 7H_2O$	2 mmol l^{-1}	Transphosphorylase activity; cell and organelle structure (Mg); sulfydryl amino acids and vitamins (S)
Micronutrients			
Calcium	$CaCl_2$	< 1 μmol l^{-1}	Possible second messenger in signal transduction; bacterial sporulation
Copper	$CuSO_4 \cdot 5H_2O$	1 μmol l^{-1}	Redox pigments
Iron	$FeCl_3 \cdot H_2O$	2 μmol l^{-1}	Heme proteins (e.g., cytochromes)
Manganese	$MnSO_4 \cdot H_2O$	2 μmol l^{-1}	Enzyme activity
Zinc	$ZnCl_2$	5 μmol l^{-1}	Alcohol dehydrogenase activity
Nickel	$NiCl_2$	5 μmol l^{-1}	Urease activity
Molybdenum	Na_2MoO_4	0.1 μmol l^{-1}	Nitrate metabolism; vitamin B_{12}
Toxic ions			
Heavy metals (e.g., cadmium, lead, mercury, etc.)		>100 μmol l^{-1}	Toxic

bacterial glucose isomerase. The media constituents and water may contain inhibitory or toxic ions, and their levels can be limited by chelating agents naturally present in the medium (e.g., citric acid, polyphosphates), by chelating agents added as supplements (e.g., EDTA), or by ion-exchange pretreatments.

By controlling the availability of some metal ions, it is possible to control the progress of certain food fermentations. For example, low levels of manganese (of the order of parts per billion) must be maintained carefully in citric acid fermentations using *Aspergillus niger*, because manganese deficiency is a prerequisite for the overproduction of citric acid. In contrast, manganese is an important activator of lactate dehydrogenase in the production of lactic acid by homofermentative species of *Lactobacillus*. In the production of ethanol by *S. cerevisiae*, it is crucially important to maintain high levels of bioavailable magnesium to ensure maximal fermentation performance.

Major requirements for magnesium, phosphorus, and sulfur can be met by the supplementation of crude media with appropriate salts (e.g., magnesium sulfate). It is not possible to generalize the ionic requirements of food microorganisms, because of differences between strains, chelation by different media, and ionic interactions. Nevertheless, it is possible to optimize metal ion concentrations in individual fermentation media by using elemental mass balances, programed search techniques, and surface-response statistical modeling.

Sources of Growth Factors

Growth factors are organic compounds that are required in very low concentrations and that perform specific catalytic or structural roles in microbial physiology. They include vitamins, purines and pyrimidines, nucleotides and nucleosides, amino acids, fatty acids, sterols, and polyamines. An auxotroph is a microorganism that is unable to synthesize one or more

Table 7 Vitamin content of selected fermentation media

	Vitamins (mg kg^{-1})						
Medium	*Biotin*	*Choline*	*Niacin*	*Pantothenate*	*Pyridoxine*	*Riboflavin*	*Thiamine*
Dried distillers' solubles	2.9	4400	110	20		15	5.5
Blackstrap molasses	1.2	750	200	60	5	2.5	1.8
Pharmamedia	1.5	3270	83	12	16	4.8	4.0
Whey powder		2420	11	48	2.9	20	4.0
Brewers' yeast		4840	498	121	50	35	75
Wheat flour		880	62	13		1.1	5.1
Soybean meal		2673	26	15		3.3	
Corn steep liquor	0.88				19		0.88
Barley malt			50	8.6		2.9	3.7

essential growth factors, and it will not grow in fermentation media lacking them. For example, the yeast *S. cerevisiae* is auxotrophic for ergosterol and oleic acid when propagated under strictly anaerobic conditions. This is because O_2 is required for the biosynthesis of the sterols and unsaturated fatty acids that are essential for the development of the yeast cell membrane.

A relative growth factor requirement is revealed when the addition of growth factors stimulates microbial growth. Microorganisms differ greatly in their requirements for growth factors. *Lactobacillus* species are particularly fastidious, requiring a range of growth factors. Many microorganisms require vitamins in the fermentation medium, at micromolar levels. These include biotin (which serves as a cofactor in carboxylase-mediated reactions), pantothenic acid (a component of coenzyme A, which is involved in acetylation reactions), nicotinic acid (in the form of nicotinamide, which is involved in redox reactions), and thiamine (as thiamine pyrophosphate, which is involved in decarboxylation reactions). Table 7 lists the vitamin content of certain fermentation media.

Complex nutritional substrates normally provide the vitamins necessary for microbial fermentation, although some types of media may be limiting in certain vitamins. For example, beet molasses is generally deficient in biotin, and cane blackstrap molasses occasionally may be deficient in pantothenic acid and inositol. Mixtures of beet and cane molasses therefore are used to ensure adequate levels of vitamins for the optimal growth of bakers' yeast. Yeast extracts are rich sources of vitamins for use in industrial fermentations. More expensive sources include soy flour, malt sprouts, and malt extract. Some fermentations benefit from the addition of commercially available media supplements or 'foods.' For example, yeast foods based on mixtures of yeast extract, ammonium phosphate, and minerals (e.g., magnesium and zinc) may be employed in alcohol fermentationsto ensure consistent yeast activity.

Design and Preparation of Food Fermentation Media

Media Design

Several important criteria need to be considered in the design and preparation of media for food fermentations. These are summarized as follows:

1. Media supply: cost effectiveness (raw materials, transport, storage), consistency and reliability of supply, nutritional variability, world political situations, and alternative carbon and nitrogen sources.
2. Media type: liquid or solid; complex, defined, or semisynthetic; nutrient limited; uses for propagation or fermentation; and balanced or unbalanced.
3. Media properties: foaming characteristics; color/pigmentation, heat-labile components, toxic components, buffering capacity, viscosity, particulate nature, biochemical oxygen demand loading, control of redox potential, ionic interactions, and microbiological stability in storage.
4. Media treatment necessary: sterile, pasteurized, or nonsterile; separate treatments for heat-labile components; pretreatments (e.g., centrifugation, acidification, ion-exchange, clarification, prehydrolysis); ease of product recovery; and effluent treatment.

In most cases, complex, inexpensive, and readily available agriculturally derived media are employed. Such media, however, are notoriously variable from batch to batch in terms of nutritional consistency. For example, the composition of molasses varies in terms of sugar and inorganic ions according to the country of origin and the production processes. Similarly, if corn steep liquor is intended to supply a particular amino acid or growth factor at critically low levels, its concentration in each batch of media should be monitored. The maintenance of reproducible fermentations using complex, undefined, and variable media thus is fraught with difficulties.

The design of chemically defined, synthetic media allows the nutritional needs of the microorganisms to be addressed, and this can improve control over fermentation performance. For example, defined media can be designed to limit the availability of carbon, nitrogen, phosphorus, metal ions, or growth factors during fermentation, and such limitation may cause a shift in the balance between growth of the microorganism and the production of desired metabolites. Defined media are more expensive than complex media, but in certain cases may be preferred (e.g., in mycoprotein production).

Semisynthetic media also can be employed. These are mixtures of defined chemicals and nondefined complex nutrient substrates. For example, a typical medium for a lactic acid bacterial fermentation may include glucose (as the carbon and energy source), diammonium hydrogen phosphate (as the nitrogen and phosphorus source), calcium carbonate (to neutralize lactic acid), and malt sprouts (as the source of growth factors and trace elements).

The desired levels of particular nutrients in the medium depend on whether the desired product is cellular biomass or primary metabolites. For example, in molasses used as an industrial medium for the production of bakers' yeast, the levels of assimilable sugar must be kept low, by controlled incremental nutrient-delivery regimes in fed-batch processes, to prevent the repression of respiration by glucose. In contrast, however, molasses can be used for the production of potable ethanol, and in this case, the sugar levels are kept high to promote fermentative metabolism.

The method of preparation of fermentation media is influential in determining choice, and the following are important considerations in the preparation of media in bulk:

1. Composition of ingredients: quality or impurities, carbon:nitrogen ratio, batch-to-batch variability, bioavailability of metal ions, and growth factors.
2. Order of solution or suspension of ingredients: pH adjustments needed before and after sterilization, and effects of sterilization on minerals and salt precipitation.
3. Changes in the medium before inoculation: temperature, aeration, agitation, and presence of antifoams.

The most important criterion in the choice of media for industrial food fermentations is cost. The cost of the production media virtually dictates the selling price of a particular commodity. World politics may affect the price and availability of fermentation substrates, so it is advisable always to have alternative substrates on hand. The costs of media pretreatment (e.g., ion-exchange, acid–enzymatic hydrolysis, pH control, antifoams) are also significant, as are the product recovery and effluent treatment costs. Such costs, however, can be reduced by judicious approaches to media design and preparation.

Media Sterilization

The prevention of microbial contamination is fundamental to many industrial food fermentation processes. Media sterilization is the destruction or removal of all forms of microbial life from the aqueous feedstock. In industrial fermentations, components such as vessels, pipework, media, inlet air, and exhaust gases are frequently sterilized by a combination of wet-heat and filtration methods. Wet-heat methods are less expensive and more effective than dry-heat methods, and thus are employed commonly in fermentation industries to destroy unwanted microorganisms. The wet-heat sterilization conditions typically used to kill all microorganisms, including bacterial spores, are listed in Table 8. These conditions may be achieved in an autoclave in an atmosphere of saturated steam.

Table 8 Wet-heat sterilization conditions

Temperature (°C)	*Time (min)*	*Pressure (kPa)*
121	15	103.4
126	10	137.8
134	3	206.7
140	0.67	261.8

Most heat treatments of industrial fermentation media are designed to selectively kill only those microorganisms of particular concern. Pasteurization is the method commonly employed for destroying frequently encountered pathogenic bacteria. Table 9 gives the pasteurization conditions used for common food fermentation media.

Strategies for the bulk sterilization of fermentation media include *in situ* steam injection of a full charge of nonsterile medium in the fermenter, or steam conduction through attemperation jackets in agitated fermenters. Alternatively, the media and vessels may be sterilized separately before fermentation. Antifoam agents, especially those that are oil based, often are difficult to sterilize. Inert, silicone-based antifoams may be used, which, although expensive, are nontoxic toward microorganisms.

The loss of available carbon and the buildup of potentially toxic or inhibitory compounds may occur during heat treatments employed to sterilize or pasteurize growth media. For example, the excessive heating of molasses may generate undesired caramelization products, following the Maillard reaction between reducing sugars and the free amino groups in proteins. Heat also may destroy vitamins and other growth factors essential for microbial growth. Some of the problems that may be encountered during media sterilization, and their possible avoidance measures, are listed in Table 10.

Fermenter inlet and exhaust gases generally are sterilized by filtration, using either depth filters (e.g., comprising porous ceramic, granular carbon, glass fiber, or synthetic membranes)

Table 9 Pasteurization conditions for some food fermentation media

Medium	*Food product*	*Typical treatment*
Molasses	Citric acid	100 °C briefly, then acidified to pH 2.5
Molasses	Bakers' yeast	Preheated to 70 °C, then flash sterilized at 136 °C for 15–30 s
Malt wort	Beer	90–100 °C for 1 h, in the presence of hops
Milk	Yogurt (skimmed milk)	High temperature short time method (flash pasteurization) – e.g., 72 °C for 15 s; 88 °C for 1 s; 90 °C for 0.5 s; or 96 °C for 0.05 s
	Cheese (full-fat milk)	Low temperature long time method (batch pasteurization) – e.g., 63 °C or 30 min

Table 10 Problems and solutions relating to media sterilization

Problem	*Solutions*
Sugar caramelization	Sterilize sugars separately and add aseptically
Metal precipitation	Sterilize phosphate source and metal salts separately
Unsuccessful sterilization of particulate and viscous media	Ensure sufficient agitation in fermenter to achieve heat transfer
Very high initial bioburden	Good housekeeping, cleaning in place, elimination of residues, sterilization of pipework dead legs

or filtration cartridges (microfilters, or microporous membrane sheets or mats).

Inocula Preparation

The development of microbial inocula for food fermentations is important to provide sufficient amounts of viable and vital biomass to carry out large-scale production effectively. Most food fermentations, with the notable exception of traditional brewing processes, employ specifically grown inocula that are discarded at the end of the fermentation process. This avoids genetic instability and microbial contamination, and it ensures the provision of high-viability cultures. The final inoculation levels are high – often 10–20% – because this reduces the fermentation time and suppresses the growth of contaminants.

Figure 2 outlines a general scheme for the multistage buildup of inoculum biomass, from a laboratory stock culture to the amount needed to inoculate an industrial production fermenter. The total amount of biomass produced depends on the properties of the medium rather than the inoculum size, but larger inocula enable maximum growth to be achieved more rapidly. With regard to the media used for the development of inocula, transfers of inocula during multistage buildup should be made between identical or similar media. This ensures that production stage growth is simply an extension of the prior seed stage growth. If the utilization of a particular component of a production medium requires the microbial cells to become enzymatically adapted, then this substrate should be included in the medium used for the development of the inoculum to prevent deadaptation and to prolong lag phases during growth.

During inoculum development, cellular biomass is required rather than fermentation products. Therefore, the medium and the conditions must be balanced properly to encourage respiratory growth and discourage fermentative metabolism. For example, in the brewing industry, the propagation of seed yeast should be conducted aerobically with sugar limitation (preferably in fed-batch mode), to ensure that sufficient conditioned biomass is produced before the commencement of anaerobic alcoholic fermentation.

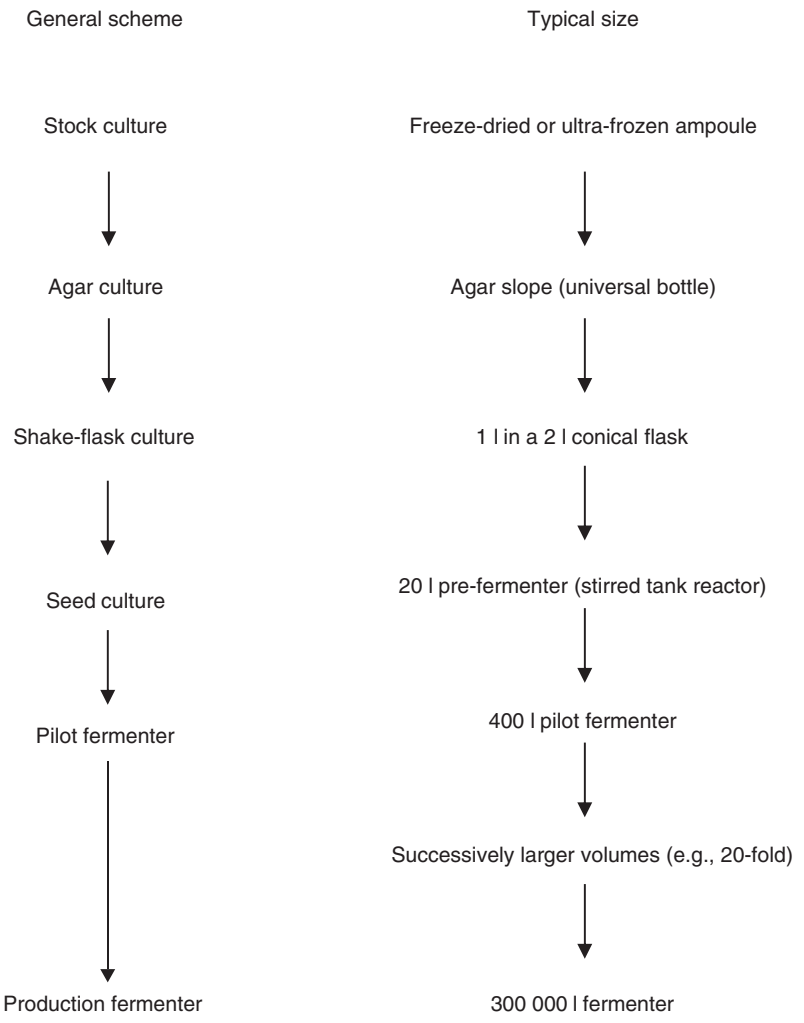

Figure 2 Inoculum preparation scheme.

See also: **Fermentation (Industrial):** Basic Considerations; ***Lactobacillus:*** Introduction; ***Saccharomyces:*** *Saccharomyces cerevisiae*.

Further Reading

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