ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 2 Issue 12 December 2019

Review Article

Production of Microbial Industrial Enzymes

Francois Niyongabo Niyonzima*

Department of Biotechnologies, Faculty of Applied Fundamental Sciences, INES-Ruhengeri, Musanze, Rwanda

***Corresponding Author:** Francois Niyongabo Niyonzima, Department of Biotechnologies, Faculty of Applied Fundamental Sciences, INES-Ruhengeri, Musanze, Rwanda.

Received: October 17, 2019; **Published:** November 11, 2019

DOI: 10.31080/ASMI.2019.02.0434

Abstract

Microbial enzymes are biological catalysts due to their ability to favor reactions more quickly and more efficiently. Various enzymes are produced by microorganisms for industry uses. They must possess the desired properties like diversified functionality, and stability over a pH and temperature ranges. The microorganisms have to produce extracellular enzymes in higher amounts and the produced enzymes have to be safe, stable, and more active. The microbial enzymes with the desired properties can be produced by optimizing fermentation conditions. To make the fermentation cost-effective, the utilization of low cost substrates such as agricultural and spent residues for microbial enzymes production is necessary. Some industrial enzymes used together for the same purpose (like amylase, lipase and protease used in detergent formulation) are co-produced together in a single fermentation to reduce the cost and to maintain the enzyme stability. In addition, for some microorganisms, recombinant DNA technology is used as an alternative strategy for overproducing huge amounts of microbial enzymes with improved substrate specificity and stability. Furthermore, novel techniques like genetic fusion of coding open reading frames or connection of proteins in a post translational process are used to manufacture the fused industrial enzymes having combined properties of their parental molecules. The public and private companies have thus to work together with academicians in order to overproduce microbial enzymes needed by the industries. The present article reviews the production of industrial enzymes and optimization of culture and fermentation conditions in order to produce microbial enzymes in huge amounts.

Keywords: Industrial Enzyme; Fermentation; Optimization; Production; Microbial Enzyme

Introduction

Enzymes are considered as wonderfully specific and efficient biocatalysts for a large number of biochemical reactions. They are not toxic and generate few byproducts, compared to chemical catalysts. Industrial enzymes with specific characteristics can be obtained from microorganisms by optimizing process parameters and by enzyme engineering. Various enzymes such as proteases, amylases, cellulases, and lipases were engineered in order to work under industrial conditions. This is necessary as wild microbial strains produce a lesser amount of enzyme, compared to engineered microorganisms. This can be achieved using special methods like use of mutagens [1,2].

The industrial enzymes are drived from plants, animals, and microorganisms. However, the microorganisms are in use to produce these enzymes owing to an important yield obtained from them, and reduction of cost and labor. Most of the industrial enzymes are secreted by Bacillus [3-16] and Aspergillus species [17-27]. Some industrial enzymes having same application can be co-produced in a single fermentation medium. In this case, the production process becomes cost effective and the enzyme stability is assured. The role of medium composition optimization is to maintain the balance between the different medium ingredients thereby preventing the amount of unused components at the end of fermentation process [28]. Kumar and Takagi [29] reported that there is no specific growth medium for the optimum industrial enzyme production by bacteria or fungi. Each bacterial or fungal species has its own growth conditions to produce industrial enzymes in a significant amount. According to Hajji*., et al*. [30], the growth medium for industrial enzymes production by bacteria and fungi is mainly optimized with the one parameter at a time method. Statistical methods are also employed to produce the industrial enzymes in adequate amounts.

Industries are still searching for new microbial strains with desired aspects in order to produce various industrial enzymes to

fulfil the current enzymes demand. The proper selection of different industrial microorganisms and the optimization of fermentation conditions is thus necessary to produce inexpensive industrial enzymes. The production of microbial industrial enzymes under optimized conditions to get enzymes with desirable properties is a continuous exercise. The concomitant production of industrial enzymes in a single-economic production medium from microorganisms is also a new challenge. Although some important works has been reported for the production and optimization of industrial enzymes from microorganisms, there is no specific report for the industrial production and optimization of culture conditions for maximum industrial enzyme production. The present review therefore reports the production and optimization of culture and fermentation conditions to produce industrial enzymes from microorganisms in important amounts. The isolation and identification of industrial enzyme-producing microorganisms were first described.

Isolation and screening of industrial enzyme-producing microorganisms

The industrial enzyme-producing microorganisms are generally isolated from soil samples collected from various places [4-9,11-13,15-17,18,20-22,24,26,27,31-35]. They are also obtained from plant bark [25]), watery environment [36,37], skim milk [3]), marine sediment [38-41], municipal solid wastes [14] and from grapes [42] (Table 1). The microorganisms producing industrial enzymes are usually screened from samples by serial dilution followed by spread plating on specific agar medium depending on the enzyme of interest to be produced by microorganisms (Table 1). Therefore, the selected isolation and screening medium contains an industrial enzyme inducer. For instance, the laccase production by *Scytalidium lignicola* was stimulated by $CuSO_4$ and $MgSO_4$ [43]. Some proteases may require casein, skim milk or gelatin as inducer [3,8,21,33,36,37,39]. The amylases, cellulases, and lipases may require starches [4,14,15,31,34,44,45], carboxymethylcellulose [13,32,35,46,47], and oil substances as inducers [32,41], respectively (Table 1).

Enzyme produced	Microorganism	Source of isolation	Screening medium	Identification	Reference
Lipase	*Acinetobacter calcoaceticus* 1-7	soil	olive oil agar	16S rDNA analysis	[32]
Protease	*Aspergillus niger*	soil	casein agar	cultural and microscopic characteristics	[19]
Pectinase	*Aspergillus niger*	soil	pectin agar	cultural and morphological characteristics	[20]
Phytase	*Aspergillus niger*	rhizosphere soil	phytate agar	18S rRNA sequence	[22]
Tannase	*Aspergillus niger*	plant barks	tannate agar	morphological and microscopic features	[25]
Cellulase	*Aspergillus awamori*	wood chips soil	carboxy methyl cellulose (CMC) agar	18s rRNA sequencing	[27]
Keratinase	*Aspergillus niger* / *flavus*	poultry farm soil	feather keratin agar	morphological and microscopic features	[18]
Invertase	*Aspergillus sojae* JU12	sugarcane soil	PDA	18S rRNA molecular sequencing	[26]
Tannase	*Aspergillus* sp. GM4	laboratory	tannate agar	morphological and microscopic features	[23]
Protease	*Aspergillus terreus* gr.	potato grown soil fields	casein agar	morphological and microscopic features	[21]
Tannase	*Aspergillus* tubingensis CICC 2651	soil	tannate agar	morphological and microscopic features	[24]
Protease	*Aspergillus versicolor* PF/F/107	poultry farm soil	Reese media	morphological and microscopic features	[17]
Protease	*Bacillus cereus*	skim milk	nutrient agar and skim milk	16S rRNA analysis	[3]

Pullulanase	*Bacillus cereus*	baking wastes, soil, and food wastes	soluble pullulan agar	morphological and biochemical experiments	[11]
Amylase	*Bacillus cereus* GA6	soil	starch agar	16S rRNA analysis	[7]
L-glutaminase	*Bacillus cereus* FT9	soil	L-glutamine agar	16S rRNA analysis	[12]
L-glutaminase	*Bacillus cereus* LC13	marine soil	minimal glutamine agar	16s rRNA analysis	[16]
CGTase	*Bacillus flexus* MSBC 2	corn field soil	soluble starch agar	16s rRNA analysis	[15]
Lipase	*Bacillus flexus* XJU-1	potato field soil	tween 80 agar	16S rDNA analysis	[6]
CGTase	*Bacillus halodurans*	sugarcane soil	soluble starch agar	microscopic examination and biochemical tests	[31]
Pullulanase	*Bacillus halodurans*	soil	pullulan agar	microscopic examination and biochemical tests	[5]
Phytase	*Bacillus lehensis* MLB2	bean-grown soil	phytate agar	morphological and physiological aspects	[10]
Amylase	*Bacillus licheniformis* AS08E	soil	starch agar	16S rDNA analysis	[34]
Protease	*Bacillus licheniformis* KBDL4	soda lake water	casein agar	16S rDNA analysis	[36]
Keratinase	*Bacillus megaterium*	poultry dump yards soil	feather meal agar	microscopic examination and biochemical tests	[59]
Cellulase	*Bacillus megaterium* BMS4	soil	carboxymethylcellulose agar	16S rDNA analysis	[35]
Lipase	*Bacillus smithii* BTMS 11	marine sediment	ZoBell's marine agar	sequence analysis	[40]
Lipase	*Bacillus sonorensis*	marine clams	olive oil agar	sequence analysis	[41]
Amylase	*Bacillus* sp.	municipal solid wastes	starch agar	16S rDNA analysis	[14]
Protease	*Bacillus* sp. PX6	soil	skim milk agar	VITEK 2 bacteria identification system	[8]
Cellulase	*Bacillus* sp., MSL2	rice paddy field soil	carboxymethyl cellulose agar	16S rDNA gene	[13]
Pectinase	*Bacillus subtilis*	soil	pectin agar	culture and biochemical aspects	[9]
Amylase	*Bacillus subtilis* AS-SO1a	soil	starch agar	16S rRNA analysis	[4]
Amylase	*Chryseobacterium* sp.	municipal solid wastes	starch agar	16S rDNA analysis	[14]
Xylanase	*Fusarium* sp. XPF-5	soil rich in plant dead material	xylan	morphological and microscopic features	[64]
Tannase	*Mucor circinelloides* F6-3-12	soil	tannic acid agar	18S rRNA gene sequencing	[63]
L-asparaginase	*Mucor hiemalis*	soil	L-asparagine agar	morphological and microscopic features	[56]
β-amylase	*Penicillium nigricans*	forest soil	starch agar	morphological and microscopic features	[45]

Cellulase	*Penicillium* sp.	soil polluted with effluents	cellulose lactose agar	morphological and microscopic features	[47]
Cellulase	*Pseudomonas fluorescens*	soil	CMC agar	morphological examination and biochemical characterizations	[46]
Amylase	*Pseudomonas stutzeri* AS22	soil	starch agar	16S rDNA analysis	[44]
Invertase	*Saccharomyces cerevisiae*	grapes	sucrose agar	biochemical tests and microscopic studies	[42]
Protease	*Scopulariopsis* sp.	poultry yards soil	skim milk agar	morphological and microscopic features	[33]
Laccase	*Scytalidium lignicola*	soil	guaiacol and 2,2'-azinobis-(3- ethylbenzthiazoline-6-sulfonate) (ABTS) agar	morphological and microscopic features	[43]
Xylanase	*Sphingobacterium* sp. SaH-05	farmland environment	xylan agar	16s rRNA analysis	[61]
Laccase	*Stereum ostrea*	wood logs	peptone glucose agar	morphological and microscopic features	[65]
Amylase	*Streptomyces* sp. A3	marine sediment	starch agar	16S rDNA analysis	[38]
Protease	*Trichoderma estonicum*	mangrove sediment	glucose yeast peptone amended with 0.4% gelatin	morphological and microscopic characteristics	[39]
L-asparaginase	*Trichoderma viride* Pers: SF Grey	marine soil	L-asparagine agar	morphological and microscopic features	[57]
Protease	*Virgibacillus* sp. EMB13	sea water	gelatin agar	16S rDNA analysis	[37]

Table 1: Isolation, screening and identification of industrial enzyme-producing microorganisms.

The formation of clear zone of specific substrate hydrolysis on agar medium plates around the microorganism is an indication of an industrial enzyme secretion by the microorganism. For example, the industrial microbial amylases produce clear zones after starch hydrolysis [14,45]. Similarly, a clear zone of casein/gelatin hydrolysis on agar plates around the organism is observed in the case of protease [3,8,21,33,36,37,39]. Furthermore, the tributyrin / Tween 80 hydrolysis or color change from red to yellow for oil-phenol red agar plate or appearance of orange fluorescent halos around industrial microorganism under UV light at 350 nm, is seen for industrial lipase secreted by microorganisms [6,32,40,41,48]. The color change is due to the release of fatty acids from oil hydrolysis that lowers the medium to the acidic pH (yellow color) [49]. The appearance of orange fluorescent halos occurred as a result of the complexation between the Rhodamine B and the oil hydrolysis products (fatty acids, mono- and diglycerides) [50].

To visualize clearly the hydrolysis zones, the culture Petri plates may be flooded with 0.1% Congo red solution followed by 1 M sodium chloride solution in the case of cellulase [13,46], saturated aqueous ammonium sulfate [39] or 10% trichloroacetic acid (TCA) or 15% of HgCl2 [21,51-53] in the case of protease, the Gram's iodine solution in the case of amylases [54,55], and phenol red that gets converted into pink color in the case of L-asparaginase [56,57]. When isolating and screening industrial enzymes, nystatin is added to the culture medium before being poured in to Petri plates to prevent fungal growth, whereas tetracycline is added to the plates for bacterial growth prevention. To isolate alkalophilic industrial enzymes-producing microorganisms, sodium carbonate is generally added to the growth medium [6,21,33]. Indeed, sodium carbonate serves as a major source of alkalinity in natural environments and when supplemented to the culture medium, the alkalophilic microorganisms used in industries under basic conditions are produced [58].

Identification of industrial enzyme-producing microorganisms

The identification of industrial enzyme-producing microorganisms, especially those which are non-toxic to human beings, are of high strategic interest. The industrial enzymes-producing bac-

teria are identified by morphological, cultural, microscopic, and biochemical characteristics [5,8-11,31,40,41,59] as cleared in the Bergey's manual of determinative bacteriology [60]. The identity is further verified and confirmed by molecular techniques such as 16S rRNA [3,4,7,12,15,16,61] or 16S rDNA sequence analysis [13,14,33-38,44] (Table 1). The industrial enzymes-producing fungi are traditionally identified on the basis of cultural, morphological and microscopic features [17-21,23-25,33,42,43,45,47,56,57,62]. The identity is further confirmed by 18S rRNA [22,26,63] sequence analysis in some cases (Table 1). Unlike for bacteria where the genomes of several bacteria have been sequenced, the fungal sequence analysis is at initial stages for fungi producing industrial enzymes. After rRNA / rDNA bacterial gene sequencing or 18S rRNA fungal molecular sequencing, the genomes have showed several new genes, and most of them were coding for industrial enzymes.

Production of microbial industrial enzymes by microorganisms

Industrial enzymes are enzymes used by various industries for commercial purpose. Production of industrial enzymes by microorganisms is a necessary step in industrial sectors. The production of industrial enzymes from microorganisms involves various steps. These are isolation, screening and identification of enzymes-producing microorganisms, optimization of process parameters and fermentation for industrial enzyme production, purification and characterization of purified enzymes, industrial enzymes formulation for sale, customer liaison, and working with the regulatory authority bodies (Figure 1). Most of the bacteria and fungi used to produce industrial enzymes are genetically modified to overproduce them in significant amount [22,66]. Solid state and submerged fermentation are often used to produce industrial enzymes. However, the submerged fermentation was repeatedly reported to be the method of choice for industrial enzymes secretion from microorganisms owing to the extracellular nature of the industrial enzyme that gets liberated in to the production medium. Sarrouh., *et al.* [66] reported that pH and temperature stability, specificity, influence of activators and inhibitors, and reaction velocity are some of the criteria used in the selection of the industrial enzymes to be produced by microorganisms.

Industrial enzymes are generally produced under carefully controlled conditions by fermentation using microorganisms, especially bacteria or fungi. *Bacillus* and *Aspergillus* species were reported to be the main producers of industrial enzymes (Table 2 and 3). Indeed, most of the species of these genera are safe and not producing any toxin, grow on inexpensive substrates and secrete extracellularly adequate amounts of enzymes in a reasonable time period. They can also be genetically manipulated easily to give novel industrial enzymes with desirable characteristics [67]. The microorganisms belonging to the *Acinetobacter, Pseudomonas, Staphylococcus, Streptomyces, Fusarium, Mucor, Penicillium*, and *Trichoderma* species are also used for industrial enzymes production (Table 2 and 3).

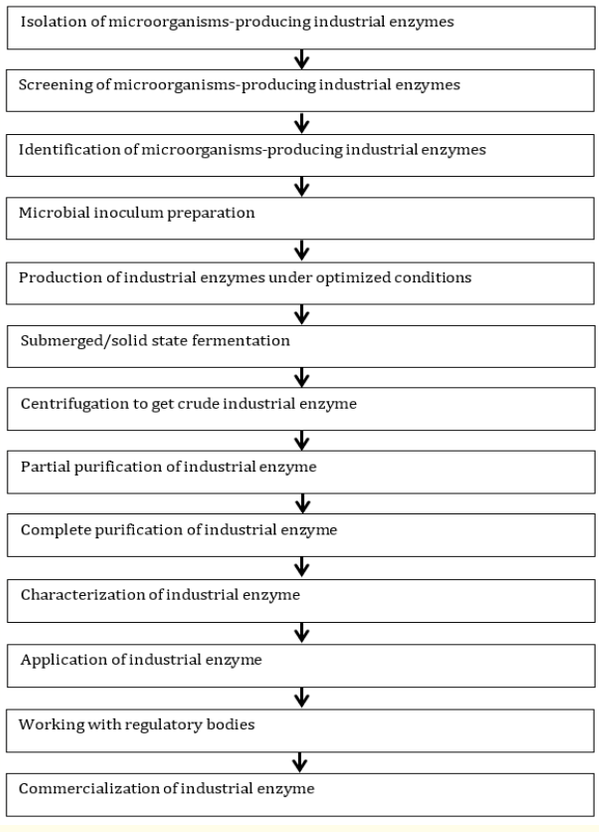

Figure 1: Production of industrial enzymes by microorganisms.

For the industrial production of microbial enzymes, submerged fermentation (SMF) and/or solid state fermentation (SSF) are employed. Each fermentation has its advantges and disadvantages. Indeed, ssubmerged fermentation is often utilized to produce industrial microbial enzymes [3,9-16,21-25,27,33,36,37,68]. It allows extracellular industrial enzyme secretion in important amounts in the production medium and thus industrial enzyme recovery is high. In addition, the culture parameters are easily controlled. However, one of the disadvantages of SmF is that it uses expensive synthetic media [6,40].

Solid-state fermentation is also used for some industrial enzymes production [26]. It uses inexpensive substances like agro-industrial by-products and downstream process is not expensive.

Substrate used	Enzymes produced	Microorganism	Reference
Chicken feathers	Keratinase	*Bacillus megaterium*	[59]
Jamun leaves	Tannase	*Aspergillus* sp. GM4	[23]
Molasses	Neutral invertase	*Aspergillus* sp	[71]
Orange peel moistened with molasses	Invertase	*Aspergillus sojae* JU12	[26]
Organic kitchen wastes	Amylase	*Chryseobacterium* sp. *Bacillus* sp.	[14]
Rice bran	Phytase	*Bacillus lehensis* MLB2	[10]
Rice bran and wheat bran	Laccase	*Stereum ostrea*	[65]
Saw-dust	Cellulase	*Penicillium* sp.	[47]
Sugarcane bagasse	Cellulase	*Aspergillus awamori*	[27]
Sugarcane bagasse or straw, wheat bran, dry corn, sawdust	Cellulase	*Pseudomonas fluorescens*	[46]
Tea stalks	Tannase	*Aspergillus tubingensis* CICC 2651	[24]
Wheat bran	Keratinase	*Aspergillus niger/flavus*	[18]
Wheat bran	Phytase	*Aspergillus niger*	[22]
Wheat bran	Xylanase	*Sphingobacterium* sp. SaH-05	[61]

Table 2: Low cost substrates used during industrial enzymes production by microorganisms.

Microorganism	Enzyme produced	pH	T(°C)	Agitation (rpm)	Inoculum size (%)	Incubation period	Good C source	Preferred N source	Reference
Bacterial species									
Acinetobacter calcoaceticus 1-7	Lipase	9	37	180	0.6	48 h	starch, olive oil,	corn flour bean flour	[32]
Bacillus cereus	Protease	10	37	n s	2	48 h	glucose and skim milk	peptone and yeast extract	[3]
Bacillus cereus	Pullulanase	6	50	200	ns	48 h	soluble pullulan	tryptone	[11]
Bacillus cereus GA6	Amylase	10	20	120	1	96 h	glycerol	ammonium acetate	[7]
Bacillus cereus FT9	L-glutaminase	7	37	150	ns	24 h	malt extract	peptone	[12]
Bacillus cereus LC13	L-Glutaminas	7	37	100	ns	24 h	maltose	L-glutamine	[16]
Bacillus flexus MSBC 2	CGTase	10	37	ns	ns	120 h	corn starch	peptone with yeast extract	[15]
Bacillus flexus XJU-1	Lipase	11	37	100	2	36 h	cotton seed oil	yeast extract	[6]
Bacillus halodurans	CGTase	10.5	37	ns	ns	48 h	Soluble starch	yeast extract and peptone	[31]
Bacillus halodurans	Pullulanase	10	37	ns	ns	72 h	pullulan	peptone	[5]
Bacillus lehensis MLB2	Phytase	5.5	30	100	2	24 h	rice bran	potassium nitrate	[10]
Bacillus licheniformis AS08E	Amylase	ns	45	200	ns	60 h	ns	ns	[34]
Bacillus licheniformis KBDL4	Protease	10	37	200	3	48 h	casein	yeast extract	[36]
Bacillus megaterium	Keratinase	10	37	ns	ns	72 h	feather meal	feather meal supplemented with yeast extract	[59]

Bacillus megaterium BMS4	Cellulase	7	37	120	ns	24 h	sucrose	LB medium BHM medium	[35]
Bacillus smithii BTMS 11	Lipase	8	28	170	3	24 h	glucose, sesame oil	soybean meal	[40]
Bacillus sonorensis	Lipase	ns	40	150	1	48 h	olive oil	peptone and yeast extract	[41]
Bacillus sp.	Amylase	7	50	120	2	48 h	soluble starch	peptone	[14]
Bacillus sp. SMIA-2	Protease	8.5	50	150	n s	36 h	starch	whey protein and corn steep liquor	[68]
Bacillus sp., MSL2	Cellulase	6	50	200		48 h	carboxymethyl-cellulose	yeast extract	[13]
Bacillus subtilis	Pectinase	8.5	37	ns	ns	24 h	glucose	yeast extract	[9]
Bacillus subtilis AS-SO1a	Amylase	6	35	150	ns	60 h	starch	beef extract	[4]
Chryseobacterium sp.	Amylase	5	50	120	2	60 h	glucose	peptone	[14]
Pseudomonas fluorescens	Cellulase	10	40	ns	ns	24 h	glucose	ammonium sulphate	[46]
Pseudomonas stutzeri AS22	Amylase	8	30	200	ns	24 h	potato starch	yeast extract	[44]
Sphingobacterium sp. SaH-05	Xylanase	8	37	150	ns	24 h	wheat bran	ammonium sulfate	[61]
Staphylococcus arlettae JPBW-1	Lipase	8	35	100	10	48 h	soybean oil	ns	[76]
Streptomyces sp. A3	Amylase	9	45	200	2	168 h	maltose	peptone	[38]
Virgibacillus sp. EMB13	Protease	8	30	150	4	24 h	mannitol	peptone and yeast extract	[37]
Fungal species									
Aspergillus niger	Pectinase	4.5	30	150	ns	7 days	pectin	peptone	[20]
Aspergillus niger	Phytase	5	30	200	ns	4 days	glucose	ammonium nitrate	[22]
Aspergillus niger	Tannase	4	37	120	ns	4 days	tannate	yeast extract	[25]
Aspergillus awamori	Cellulase	6	25	ns	ns	7 days	carboxy methyl cellulose (CMC)	peptone	[27]
Aspergillus niger / flavus	Keratinase	8.5	37	120	ns	4 days	keratin	peptone	[18]
Aspergillus sojae JU12	Invertase	8	37	ns	9	5 days	orange peel moistened with molasses	beef extract	[26]
Aspergillus sp. GM4	Tannase	4	30	ns	ns	4 days	jamun leaves / tannate	potassium nitrate	[23]
Aspergillus terreus gr.	Protease	10	37	n s	2	5 days	casein	soybean meal	[21]
Aspergillus versicolor PF/F/107	Protease	9	35	150	n s	4 days	wheat bran	sodium nitrate	[17]
Fusarium sp. XPF-5	Xylanase	8	47	160	ns	4 days	xylan	ns	[64]
Mucor circinelloides F6-3-12	Tannase	5.5	30	200	2	7 days	green tea leaves powder	Sodium nitrate	[63]

Mucor hiemalis	L-asparagi-nase	7	30	ns	ns	4 days	glucose	L-asparagine	[56]
Penicillium nigri-cans	**β-**amaylase	5	60	ns	ns	ns	starch	sodium nitrite	[45]
Penicillium sp.	Cellulase	5	30	ns	ns	7 days	lactose	yeast extract	[47]
Saccharomyces cerevisiae	Invertase	7	30	ns	ns	2 days	starch	urea	[42]
Scopulariopsis sp.	Protease	9	37	n s	3	5 days	casein	tryptone	[33]
Scytalidium lignicola	Laccase	6	30	ns	ns	7 days	sucrose	sodium nitrate	[43]
Stereum ostrea	Laccase	5.5	30	180	ns	12 days	glucose	peptone	[65]
Trichoderma viride Pers: SF Grey	L-asparagi-nase	6.5	37	ns	ns	3 days	maltose	L-asparagine and peptone	[57]

Table 3: Optimum conditions for industrial enzymes produced by bacteria and fungi ns: not specified / not determined.

In addition, the higher industrial enzyme yields is obtained in a brief period of time. However, the physicochemical parameters are not easily monitored and regulated [32]. Some industrial enzymes were reported to be produced by both solid and submerged fermentation. For instance, the invertase (β D fructofuranosidase) was produced by *Aspergillus sojae* JU12 using SSF and the fermentation was cost effective as orange peels moistened with molasses were used. The enzyme is very important in the production of alcoholic beverages [26]. Similarly, the abundant agricultural residue known as lignocellulosic biomass was used to produce cellulase in significant amount with *Aspergillus awamori* [27].

Batch and fed batch fermentation, with constant and/or linear feeding, are also used for industrial enzyme production by microorganisms. Aishwarya., *et al.* [69] reported the production of a detergent protease by the bacterium *Alcaligenes* sp. (MTCC 9730) using fed batch fermentation. Different statistical experimental designs are used to produce industrial enzymes by microorganisms in higher amount. For instance, a sequential statistical strategy, by Plackett–Burman design followed by steepest ascent method and response surface methodology, was used to maximally produce tannase from *Aspergillus* [24]. Plackett-Burman design was used in SSF to produce a tannase by *Aspergillus* sp. GM4 [23].

The production of some industrial enzymes is stimulated by various substances in the production medium. For instance, the production of the detergent lipase by *Bacillus flexus* XJU-1 was activated by Tween-80 and Triton X-100. Indeed, the present surfactants act by modifying plasma membrane thereby activating the medium compounds uptake, leading to the lipase release in a significant amount [6,54]. Prasanna., *et al.* [47] reported the increase in extracellular cellulase secretion by *Penicillium* sp. when the surfactant Triton-X100 was supplemented to the fermentation medium. The production of laccase by a mushroom *Stereum ostrea* was stimulated by various inducers such as aromatic or phenolic compounds, copper and surfactants. Indeed, the micronutrient copper activates laccase transcription and production, whereas surfactants favor enzyme production by stimulating spores growth and enhancing the availability of less soluble substrates for the microorganism [65].

The use of the inexpensive by-products or agricultural residues does not make only the fermentation and production cost effective, but also reduces the environmental pollution that may be caused by by-products or agricultural residues disposition. Indeed, if a cheap substrate is chosen carefully to grow the microorganism, one third of process cost can be reduced [70]. For instance, keratinase was produced in a significant amount by *Bacillus megaterium* when chicken feathers were used as both carbon and nitrogen sources [59]. Similarly, Lincoln and More [71] optimally produce invertase from *Aspergillus sojae* JU12 when orange peel moistened with molasses were used as substrates. Likewise, wheat bran, a cheap agricultural substrate, was used by different *Aspergillus* species to produce phytase [22], keratinase [18], and laccase [65]. The coconut cake was also reported to be the best substrate for cellulase secretion by *Pseudomonas fluorescens* [46]. The table 2 shows some by-products or agricultural residues which are used as substrates during industrial enzymes production by microorganisms.

To meet the present increased industrial enzymes demand, the concomitant production of some enzymes by microorganisms in a single cultivation medium with cheap substrates becomes necessary. Industrial enzymes have been concomitantly produced from microorganisms and used in industries. Indeed, if two, three, or

more industrial enzymes are coproduced by bacteria or fungi under similar conditions, the whole process is not expensive. In addtion, the stability among the simultanesously produced enzymes is assured. For example, protease, lipase, amylase, and cellulase are used in detergent industries to remove variious stains. If they are produced by the same cultivation medium, the proteolysis of lipase, amylase, and cellulase by the protease is prevented. No amylase or lipase proteolysis observed when the protease, lipase and amylase were coproduced together by *Bacillus flexus* XJU-1 in the same fermentation [54]. The amylases, cellulases, and lipases resistant to protease hydrolysis have an excellent applicability to the detergent formulation. The alkaline amylase of *Bacillus megaterium* B69 was also not hydrolyzed by the alkaline protease when concomittently produced together [72].

The agro-industrial byproducts were also used as inexpensive substrates for the simultaneous production of industrial enzymes in higher amounts. Shivakumar [73] coproduced an important amounts of amylase and protease by *Bacillus* sp. Y using cheap substrates under solid state fermentation. Similarly, the agro-industrial waste mustard oilseed cake was the substrate of choice for amylase and protease coproduction by *Bacillus megaterium* B69 [72]. Likewise, the inexpensive substrate was employed for the concomitant production of lipase and amylase by Bacillus subtilis JPBW-9 [74]. The use of the agricultural residue or byproducts as production substrates to produce industrial enzymes by microorganisms makes the fermentation process cost effective, and also avoid environmental pollution by using these wastes.

Optimization of process parameters for maximum industrial enzymes production by microorganisms

The industrial enzymes production by microorganisms is principally influenced by various factors such as incubation time, agitation/shaking, initial pH, inoculum concentration, incubation temperature, carbon source, metal ions, and nitrogen source (Table 3). The optimization of these process factors has a significant role in enhancing the industrial enzyme yield. The optimization of media components, cultural parameters and fermentation conditions is therefore necessary to maximally produce the industrial enzymes in adequate amounts. The process factors are generally optimized one factor each time, holding all other factors unchanged and the optimized condition/factor is taken into account in the subsequent experiments in sequential order. The advantage of the optimization of various nutritional parameters, physico-chemical aspects, and fermentation factors is that it helps in designing a cost effective fermentation process [21,33,34,36,75].

The optimization of the process parameters by statistical methods are also used to increase the production of industrial enzymes in a shorter time. For instance, nutritional factors (C, N, and P sources) and physico-chemical parameters (inoculum age, incubation time and temperature) were optimized by applying Plackett-Burman design and the Box–Behken design and the enhancement in tannase production by *Mucor circinelloides* F6-3-12 was observed [63]. An adequate amount of lipase by was produced by *Staphylococcus arlettae* JPBW-1 using response surface methodology (RSM) [76]. Roy and Mukherjee [34] use *Bacillus subtilis* DM-03 and *Bacillus licheniformis* AS08E to maximally produce the amylases with statistical methods [34]. The culture conditions for the production of protease by *Trichoderma estonicum* was optimized by a 2-level factorial Plackett-Burman design followed by central composite design [39]. A list of various microorganisms producing industrial enzymes and the optimum conditions for the industrial enzymes production are shown in the table 3.

Time course study for the industrial enzymes production by microorganisms

The incubation time plays an important role in the production of industrial enzymes by bacteria and fungi. The optimal time recorded for industrial enzymes production mainly ranged from 24 to 48 h for bacterial species [6,9-14,16,31,35,36,37,40,41,44,46,61,68,76]. The shorter incubation time reported for most of industrial enzymes production makes the fermentation process inexpensive. The incubation period of 60-96 h range was also reported for the production of industrial enzymes such as keratinase, protease, pullulanase, amylase and lipase by bacteria [5,7,14,34,59]. A higher incubation period of 120 and 168 h were observed for cyclodextrin glycosyltransferase (or CGTase) production by *Bacillus flexus* MSBC 2 [15] and amylase secretion by Streptomyces strain A3 [38], respectively (Table 3). Therefore, the time period of industrial enzymes secretion by bacteria varies from one species to another. This may be ascribed to the genomes difference.

The fungal industrial enzymes are secreted at optimal level at 4 or 5th day [17,18,21-23,25,26,33,56,64] (Table 3). A low fermentation time of 2 and 3 days was observed for the production of the invertases from the *Saccharomyces cerevisiae* [42] and L-asparaginase by *Trichoderma viride* Pers: SF Grey [57], respectively. The higher incubation time of 7 days was also noted for the production of pectinases by *Aspergillus niger* [20], cellulases by *Penicillium* sp. and *Aspergillus awamori* [27,47], tannase by *Mucor circinelloides* isolate F6-3-12 [63], and laccases by *Scytalidium lignicola* [43]. Likewise, Usha., *et al.* [65] reported a higher incubation time of 12 days when laccase was secreted in an adequate amounts by *Stereum ostrea*.

In general, as the incubation time increases, the industrial enzymes secretion by microorganisms also increases. However, after optimum incubation period, a decline in industrial enzyme production is observed. This decrease in industrial enzymes production was attributed to the reduced availability of nutrients and the toxic metabolites secretion [77] or decomposition of industrial enzyme by the protease [78]. For industrial lipase, the decrease was ascribed to the accumulation of fatty acids and glycerol resulted from lipolysis [79]. For most industrial enzymes like amylases, the decrease in amylase production was due to the enzyme denaturation resulted from the enzyme produced and the medium components interaction [52,53]. The variation in industrial enzyme production by bacteria and fungi can be attributed to growth and metabolic activities variation [53,80].

Influence of initial pH of the medium on industrial enzymes production by microorganisms

The initial pH of the culture and fermentation medium is a major factor regulating industrial microbial enzymes secretion. It may influence the availability of nutrient substrates or the transport of various nutrient components across the bacterial or fungal membranes, which in turn stimulates the microbial growth and thus industrial enzymes production [53,75,80]. The optimum initial pH range recorded for most of the industrial bacterial enzymes is the 6 to 10 range [5-7,9,11-13,15,16,34,35-38,40,41,44,46,59,61,68,76]. A low pH of 5.0 and 5.5 was seen for amylase production by Chryseobacterium sp. [14] and phytase by *Bacillus lehensis* MLB2 [10], respectively. 10.5 was higher pH recorded for industrial CG Tase production by *Bacillus halodurans* [31] (Table 3). The difference in genomes may also be the reason why the bacteria producing industrial enzymes have different initial pH requirements.

The optimum pH observed for fungal industrial enzymes production ranges from acidic to basic pH range, viz. pH 5 to 9 [17,18,25-27,33,42,43,47,56,57,63-65]. Niyonzima and More [21] produced an alkaline protease active at pH of 10.0 using *Aspergillus terreus* gr. Similary, lower pH values of 4 and 4.5 were observed as optimum initial pH in the production of tannase, phytase, and pectinases by *Aspergillus* species [20,22,23] (Table 3). The variation in industrial enzyme yields at different initial pH requirement may be due to the bacterial or fungal strain specificity. Any deviation from optimum initial pH resulted in low industrial enzyme secretion. This was attributed to the disruption of transport mechanisms through the bacterial or fungal membrane that prevents the industrial enzyme release [81].

Effect of incubation temperature on the industrial enzymes production by microorganisms

The incubation temperature is a vital environmental parameter for industrial enzymes secretion by microorganisms. Like initial pH, it may influence the growth of bacteria and fungi, and thus industrial bacterial or fungal enzymes production. The optimum incubation temperature seen for bacterial industrial enzymes production is in the 30 to 50 °C range. 37 °C was noted as optimum production temperature in most cases [3,5,6,9-12,31,32,35,36,59,61] (Table 3). A low optimum incubation temperature of 20 and 28 °C were also observed for industrial amylase and lipase production, respectively by *Bacillus* species [7,40]. For fungal species, the optimum incubation temperature is ranged from 25 to 47 °C [17,18,20-23,25-27,33,42,43,47,56,57,63-65]. However, 60 °C was optimum fermentation temperature for β-amaylase production by *Penicillium nigricans* [45]. At elevated incubation temperature, the yield in industrial enzymes production is low due to the thermoliability of the industrial enzymes or the denaturing of industrial enzyme structure in the active site.

Effect of inoculum level on the industrial enzymes production by microorganisms

The concentration of inoculum is one of the key culture parameters for microbial growth and thus industrial enzymes production. Various inoculum concentrations ranging from 0.6 to 4% were optimum for bacterial industrial enzymes production by microorganisms [3,6,10,14,32,36-38,40,41] (Table 3). However, Chauhan., *et al.* [76] reported a detergent lipase production by *Staphylococcus arlettae* JPBW-1 when a higher inoculum size of 10% was used. Different inoculum levels were found to maximally produce the industrial enzymes by different fungi. For instance, the inoculum level of 2% was optimum for the tannase production by *Mucor circinelloides* isolate F6-3-12 [63] and protease production by *Aspergillus terreus* gr. [21]. 3% was inoculum size optimum for the secretion of a protease by *Scopulariopsis* sp. [33]. Lincoln and More [71] obtained an industrial invertase from *Aspergillus sojae* JU12 using a higher inoculum level of 9%. The effect of inoculum on industrial enzymes secretion may thus depend on the type of microorganism, inoculum load, and the bacterial or fungal size, and type.

Generally, an important increase in industrial enzymes production by microorganisms correlated with an enhancement in inoculum concentration till optimum inoculum size reached [21,33] owing to rapid substrate degradation [82]. Indeed, the industrial enzyme production by microorganisms is often high at lower inoculum levels; however, a low industrial enzyme yield is observed

after optimum inoculum size [83]. Hesseltine., *et al*. [84] proposed that the decrease observed when an important inoculum level is used can be attributed to the faster bacterial or fungal growth and thus shortage of the nutrients. Likewise, Hasan., *et al.* [14] observed a low yield at higher inoculum level owing to the lack of total dissolved oxygen and nutrient supply to the microorganisms.

Effect of carbon source on industrial enzymes production by microorganisms

The carbon sources serve as a primary energy source for bacterial and fungal growth and therefore industrial enzymes production. The carbon sources such as starch [4,14,15,32,68] glucose [9,14,46], soluble pullulan [5,11], malt extract [12], maltose [16,38], pullulan [5], sucrose [35], and carboxymethylcellulose [13] are used for the production of the bacterial industrial enzymes. Soybean oil [76], olive oil [41] and cotton seed oil [6] were also used as carbon sources. In some cases, a mixture of carbon sources like corn flour and bean flour [32], glucose and skim milk [3], and glucose and sesame oil [40] are used in the secretion of bacterial enzymes (Table 3).

For the production of industrial fungal enzymes, starch [42,45], glucose [22,56,65], maltose [57], sucrose [43], lactose [47], carboxymethylcellulose [27], pectin [20], and tannate [25] are employed as carbon sources. Various inexpensive substances such wheat bran [17], jamun leaves [23], green tea leaves [63], and orange peel moistened with molasses [26] were preferred as carbon sources for industrial enzymes production by fungi. The industrial enzymes are generally produced by microorganisms with low carbon source concentration. This make the production cost effective used. In some cases, a carbon source repression is obsrved when a carbon source is used in a significant amout. When an important amount of carbon source is utilized, industrial enzymes secretion decreased owing to limitation of oxygen transfer resulting in poor bacterial or fungal growth [6].

Effect of nitrogen sources on industrial enzymes production by microorganisms

The nitrogen sources served as a secondary energy source for the microbial growth and thus for industrial enzymes production. They play an important role in most of the microorganisms to synthetize the cell wall components, amino acids, peptides, proteins (including industrial enzymes), and nucleotides / nucleic acids [85]. For the production of bacterial industrial enzymes, tryptone [11], peptone [5,12,14,38], yeast extract [6,9,36,44], and beef extract [4] are employed (Table 3). Sometimes, the optimum industrial enzymes production is observed when there is combination of nitrogen sources, like peptone and yeast extract [15,31,41,68]. The cheap organic nitrogen sources like corn flour and bean flour [32] and soybean meal [40] are also utilized to produce industrial enzymes by bacteria.

Like for bacteria, organic nitrogen sources such as peptone [18,20,27,65], tryptone [33], urea [42], yeast extract [25,47], beef extract [26], and L-asparagine [56] are used in industrial fungal enzymes production. In general, the industrial enzymes are generally produced at their optimum levels when organic nitrogen sources are incorporated in the production medium. The preference of organic nitrogen sources by industrial enzymes producing microorganisms can be ascribed to the presence of some micro-and micronutrients, vitamins, amino acids and/or peptides, and growth factors present in them [33,36,37] (Table 3).

The feather meal served as both carbon and nitrogen carbon source for industrial keratinase production by *Bacillus megaterium* [59]. The nitrogen sources stimulate the industrial enzymes production up to certain level beyond which a nitrogen metabolite repression is seen. Indeed, the complex organic nitrogen sources may show industrial enzymes repression when employed in high amounts because they are rich in amino acids (such as glycine and valine) and short peptides. A higher nitrogen source concentration is therefore inhibitory to the industrial enzyme secretion by bacteria or fungi.

Although the inorganic nitrogen sources are not generally often found to increase the production of the industrial enzymes, an important industrial enzymes production was observed in some cases with them. For instance, a significant industrial enzyme production was seen when ammonium acetate [7], potassium nitrate [10], and ammonium sulfate [46,61] were used from industrial production by bacteria. Similarly, ammonium nitrate [22], potassium nitrate [23], sodium nitrate [17,43,63] and sodium nitrite [45] were inorganic nitrogen sources used to produce industrial enzymes by fungi.

Effect of agitation on the industrial enzymes production

The industrial enzymes-producing microorganisms are usually grown under shaking conditions. The production of bacterial industrial enzymes is often done with shaking in the range of 100 to 200 rev/min (Table 3). For instance, 100 rpm was the optimum for the production of industrial enzymes by *Bacillus* species [6,10,16], while 200 rpm was also best for some bacterial species [11,34,36,38,44]. Similarly, the production of industrial enzymes by fungi was agitated in the 120 to 200 rev/min range. For examples, 120 rpm [18,25], 150 [17,20], 160 [64], 180 [65], and 200

rpm [22,63] were found as optimal shaking conditions for fungal species. The agitation of the culture flasks at a moderate rate allows a good availability of the nutrients to the microorganisms and a proper aeration, favoring thus the production of industrial enzymes in important amounts.

Conclusion

In the present review, an overview was given for the production and optimization of nutritional and physical parameters of industrial enzymes. Since there is a nonstop demand of industrial enzymes, the present information can help the other researchers to optimize industrial enzymes production cost effectively. Although various industrial enzymes have been produced under optimized conditions in the last decade, most of them are not marketed. This was mainly attributed to the non-cost-effective production and lack of enzymes with desired properties. The intensive studies are still needed to get bacterial or fungal industrial enzymes with better properties. The various companies must thus work together with academic institutions to strength this linkage.

Acknowledgments

Author is thankful to INES Ruhengeri management for support.

Conflict of Interest

Author has no conflict of interests.

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