Advances in Environmental Biology, 11(2) February 2017, Pages: 113-122

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Microbial Chitinases Production Optimization Using Classical and Statistical Approach (Review)

[1,2]Ben Amar Cheba

[1]Department of Biology, College of Science, Al Jouf University, Kingdom of Saudi Arabia (KSA).
[2]Department of Biotechnology, Faculty of Nature and Life Sciences, University of Sciences and Technology of Oran -Mohamed Boudiaf (USTOMB), BP 1505 Al Mnaouar, Oran 31000, Algeria.

Address for Correspondence:
Ben Amar Cheba, Department of Biology, College of Science, Al Jouf University, Kingdom of Saudi Arabia (KSA).
Phone number 0966 536832385; E-mail: benach57@yahoo.com

Received 18 December 2016; Accepted 12 February 2017; Available online 26 February 2017

ABSTRACT

Production of microbial chitinases (EC.3.2.1.14) has received increased attention in recent years due to its versatile biotechnological applications, and is preferred source due to their low production cost, less time consuming, high productivity, and easy availability of raw cultivation substrates, but their production is greatly influenced by media nutritional components and cultivation conditions. In this context, the medium optimization studies and searching for chitinases production key factors, still an urgent need to maximize the production and meet the industrial demands. In this review, we summarize the wealth of data available in the literature regarding to the microbial production of chitinases which includes informations on potential producers, medium composition (carbon and nitrogen sources, substrate concentration, metal ions, salinity, pH,) cultivation conditions (incubation period, temperature, inoculums size, and agitation rate) and other physicochemical parameters, which are expected to improve the enzyme production and minimize the product cost.

KEYWORDS: Chitinase, microorganisms, production, medium composition, culture conditions, statistical optimization

INTRODUCTION

Next to cellulose, chitin, an insoluble biopolymer of β-(1,4)-linked N-acetylglucosamine (GlcNAc) is the most abundant biopolymer on the planet, and occurs mainly as a structural component in the cell walls of fungi, cuticles of insects and exoskeleton of arthropods (e.g., crabs, shrimps, and lobsters). It forms the major source of carbon and nitrogen for the microbial population in the aquatic biosphere [1, 2]

Despite the early discovery of chitin in1811 by Henri Braconnot, Chitinases (EC.3.2.1.14), which hydrolyze β-1, 4-glucosidic bonds of chitin was described for the first time in 1911 by Bernard in orchid bulbs [3], whereas in animals the presence of chitinase was marked in snails by [4].

Chitinases, belonging to the family of glycosyl hydrolases, are responsible for the biological conversion of chitin to N-acetyl glucosamine. The ensemble of chitinolytic enzymes can contain endochitinases (EC 3.2.1.14), exochitinases (EC 3.2.1.52), chitobiosidases (EC 3.2.1.30) and N-acetylglucosaminidases (NAGases) (EC 3.2.1.96) that are widely distributed in the biological word and have attracted a great attention due to their versatile biotechnological applications that ranging from shellfish waste management and phytopathogens control [5,6]to disease and cancer prognosis, [7,8]

Chitinases are widely distributed in the biological word; they are produced from different sources such as plant, animals and microorganisms. The microbial enzymes generally meet industrial demands, and are preferred source due to their unique properties and low production cost, but their production is noticeably influenced by medium components and environmental factors [9,10,11,12,13], for this raison, the medium optimization studies

and searching for chitinases production key factors, still an urgent need to maximize the production and meet the industrial demands. In this review, we recapitulate the wealth of data available in the literatures regarding to microbial chitinases production optimization using classical and statistical approach, which are expected to improve the enzyme production.

1- Chitinase microbial sources:

Chitinases are produced by wide range of organisms including: viruses, bacteria, archaea, actinomycetes, yeasts, fungi, plants, protozoans, animals and also in human beings [14]. the most potent producers are fungal genera such as: *Trichoderma, Penicillium, Aspergillus, Verticillium Metharhizium, Beauveria, Lecanicillium, Aphanocladium, Neurospora, Mucor, Stachybotrys, Lycoperdon, Myrothecium, Conidiobolus and Agaricus,* [15,16,17] Yeast chitinases from *Saccharomyces, Pichia, Rhodotorula* and *Cryptococcus* [18,19]. Chitinases are widely distributed in Gram negative bacteria such as *Serratia marcescens, Vibrio, Chromobacterium, Klebsiella, Pseudomonas, Xanthomonas and Aeromonas,* as well as in Gram positive one as *Bacillus, lysinibacillus, Clostridium, Arthrobacter, Nocardia* and *Streptomyces* [20,21,22]. Bacterial chitinases are thought to be important in the digestion of chitin for utilization as a carbon and energy source and from the ecological point of view, such chitinases serve as an important role in recycling chitin in nature, furthermore their production is time and cost effective [23].

2-Chitinases production optimization using classical approach:

Microbial chitinases are mostly intracellular inducible and greatly influenced by nutritional and physicochemical factors, such as carbon and nitrogen sources, pH, temperature, inorganic salts, agitation, dissolved oxygen concentration and incubation period. The major factor for the expression of chitinases activity has always been the type and the form of chitin and the most inducing substrates are colloidal chitin, crystalline chitin or shrimp or crab powder.The chitinases production of *Bacillus licheniformis x-7u* and *Bacillus sp r2* is significantly influenced by addition of 0.5% N-acetyl glucosamine and glucose, respectively [24,13] where the chitinases of *Vibrio alginolyticus H-8* and *Salinivibrio costicola strain 5SH-1* are enhanced by addition of 0.6% and 1% glucose respectively [25,26] for more details a list of various media and production conditions optimized with different microorganisms was presented in (Table 1).

Several studies on chitinases optimization have been reported earlier with effects of different media components and production conditions on its secretion using the classical approach (one-factor-at-a-time" OFAT " method).which means changing one factor at a time while keeping others at a constant level [27], however, this approach becomes laborious, expensive and it generates large quantities of data which are often difficult to interpret ,furthermore, extremely time consuming due to their requirement to large number of experiments, which are unreliable and unmanageable specially when large number of variables have to be investigated. Moreover, the most defect of OFAT approach is its inability to detect the frequent interactions occurring between the multiple factors involved. [28, 29, 30].

3-Chitinases production optimization using statistical approach:

The conventional parametric or OFAT technique is not always feasible for enzyme production due to their limitations cited above. To overcome these problems, a number of statistical methodologies are developed and designed not only to reduce the number of necessary experiments in the optimization process, which save time, chemicals and manpower, but also to produce more defined results and considers the interaction effects among the variables ,in addition to other advantages in their use such as rapidity, simplicity, reliability, short-listing of nutrients, and help to understand the effect of the nutrients at varying concentrations ,as well as determines their significance levels[31, 32, 33].

Various statistical designs are available such as full factorial, fractional factorial or Plackett–Burman design, Taguchi's robust design and response surface methodology [34]. Plackett–Burman central composite design (CCD) and Box–Behnken Response Surface Methodology are powerful statistical tools for screening and identifying significant influencing factors and optimizing medium components for enzyme production [35, 36]. There are very few reports on microbial chitinase production optimization using the statistical designs, the (Table 2) summarizes the production conditions and medium composition optimized using statistical approach.

Table 1: Chitinase production conditions and medium composition optimized using classical approach

Source/ Reference	Production medium	Production mode (I) or (C)	pH	Temperature (°C)	Shaking (rpm)	Incubation period (day)	Inducer (substrate)	Remarks
Acinetobacter sp. strain CHB 101 (37)	M 9 medium ± 0.25% chitosan with D. A of 30% or ± 0.2% glucose or glucosamin	C	5.6	25	150	7 days	--------	Chitosanase
Aeromonas schubertii (38)	0.35% K_2H $PO_{4,\ 0.1}$% Mg SO_4. $7H_2O$, 0.15% KH_2	I	4.8	28	ND	4 days	Chitin powder	---------

	PO_4, 0.1% yeast extract, 0.1% typton, 1% chitin							
Alteromonas sp. strain 0-7 (39)	Artificial sea water + 5 g/l Bactopeptone , 1 yeast extract , 5 colloidal chitin	I	7.6-7.8	27	200	1 day	Colloidal chitin	---------
Bacillus cereus (40)	M 9 medium + 0.25% colloidal chitin	I	ND	30	ND	6 days	Colloidal chitin	5distinct chitinases isoforms produced
Bacillus circulans wL-12 (23)	Yeast nitrogen base (YNB) medium (Difco) + 0.2% chitin + 0.5% yeast extract	I	ND	30	ND	1-4 days	Chitin	6distinct chitinases isoforms produced
Bacillus licheniformis X- 7u (24)	0.5% colloidal chitin 0.5% GlcNAc, 0.1% yeast extract, 0.05% $MgSO_4$, 0.31% Na_2HPO_4. $12H_2O$, 0.2%NH_4NO_3, 0.1% KH_2PO4, 0.05% NaCl, 0.01% $CaCl_2$. $2H_2O$	I	7	50	ND	2 days	Colloidal chitin	4 distinct thermostable chitinases
Bacillus pabuli K1(41)	$1.5K_2HPO_4$, $1.5KH_2PO_4$, 0.7 Mg SO_4 .$7H_2O$ .0.5 NaCl, 0.5KCl, 0.13 $CaCl_2$. $2H_2O$, 0.5 yeast extract + 0.15 colloidal chitin from crab shell (sigma)	I	8	30	200	35h	Colloidal chitin	Initial oxygen10% and a_w >0.99
Bacillus sp. BG-11 (42)	1% swollen chitin ,0.5% glucose, 025% yeast extract, 0.25% cosamino acids, 0.5% NaCl, 0.1%KH_2PO_4 , 0.01% Mg SO_4 . $7H_2O$, 0.1% (v/v) soil extract	C	8.5	50	0	3 days	Swollen chitin	Inoculum size 2%
Bacillus sp. MET 1299 (43)	L-B Broth	C	7	30	ND	1 day	---------	Chitosanase
Bacillus sp. NCTU2 (44)	M 9 medium +1% colloidal chitin	I	6.5	37	ND	3 days	Colloidal chitin	Inoculum size 1%
Bacillus sp. strain MH-1 (45)	0.5%colloidal chitin, 0.7% $(NH_4)_2$ SO_4, 0.1% K_2HPO_4, 0.1% NaCl, 0.01% MgSO4. $7H_2O$, 0.05% yeast extract, 1% compost extract + 0.03% (2,6-0- dimethyl) β-cyclodextrin (DMCD)	I		58	ND	3-4 days	Colloidal chitin	3 thermostable endochitinases
Bacillus sp. R2 (13)	75% natural sea water, 0.5% shrimp shell colloidal chitin ,0.5% glucose, 0.5% yeast extract.	I	7.5	30	180	1 days	Colloidal chitin	Inoculum size 2.5%
Bacills sp.13.26 (46)	07% $(NH_4)_2$ SO_4, 0.1% K_2HPO_4, 0.1% NaCl, 0.01% $MgSO_4$. $7H_2O$, 0.05% yeast extract ,0.1% bacto trypton + 0.5% colloidal chitin	I	7	55	120	3 days	Colloidal chitin	Inoculum size 10%
Bacillus stearothermophilus CH-4 (47)	0.2% sodium acetate, 0.6% $(NH_4)_2$ SO_4 ,0.1% K_2HPO_4, 0.1% NaCl, 0.01% $MgSO_4.7H_2O$,0.01% yeast extract +2% compost extract containing chitin	I	ND	58	ND	1 day	Chitin compost	Thermostable exochitinase
Cellulomonas flavigena NTOU1 (48)	1% shrimp shell powder, 0.1% KH_2PO_4, 05% NaCl, 0.1% pectin, 0.01% $CaCl_2$, 0.05% $MgSO_4$. $7H_2O$, 0.5% yeast extract	I	8	30	120	4-5 days	Shrimp shell powder	1% inoculum size
Enterobacter sp. NRG4 (49)	0.1% swollen chitin 0.05% yeast extract, 0.5% peptone, 0.1% KH_2PO_4 and 0.01% $MgSO_4$. $7H_2O$	I	8	30	150	3 days	Swollen chitin	---------
Pantoea dispersa	0.5% swollen chitin,	I	7.2	30 ± 2	180	144h	Swollen	5% inoculum

(31)	0.05% yeast extract, 0.1% $(NH_4)_2$ SO_4, 0.06% $MgSO_4$. $7H_2O$ and 0.136 KH_2PO_4						chitin	(10^8 CFU/ml)
Pseudomonas aeruginosa K -187 (50)	3% SCSP, 0.1% CMC, 0.1% $(NH4)_2$ SO_4, 0.1% K_2HPO_4, 0.1 $MgSO_4$. $7H_2O$, 0.1% $ZnSO_4$. $7H_2O$	I	9	45	ND	3 days	Shrimp and crab shell powder	The purified enzyme had antibacterial activity
Salinivibrio costicola strain 5S M-1 (26)	LB NaCl (1%trypton, 0.5% yeast extract + 3% NaCl) ± colloidal chitin 0.1% or 1% glucose	C	7	30	ND	1 day	--------	Constitutive enzyme

(I): inducible, (C): continuous, ND: not determined

Table 1: Chitinase production conditions and medium composition optimized using classical approach. (Continue)

Source/ Reference	Production medium	production mode (I) or (C)	pH	Temperature (°C)	Shaking (rpm)	Incubation period (day)	Inducer (substrate)	Remarks
Vibrio alginolyticus H-8 (25)	75% sea water, 0.5% squid chitin, 0.6% glucose, 0.75% peptone 0.02% K_2HPO_4, 0.2% yeast extract	I	7	30	ND	1 day	Squid chitin	---------
Vibrio carchariae (51)	Marin medium + 2.5% swollen chitin	I	7.6	30	ND	40h	Swollen chitin	---------
Xanthomonas sp. strain AK (52)	0.2% powdered chitin, 0.1% yeast extract, 0.1% polypepton	I	7	37	ND	1 day	Chitin powder	---------
Streptomyces albovinaceus S-22 (53)	0.2% colloidal chitin, 0.05% KCl, 0.1% K_2HPO_4, 0.05% Mg SO_4. $7H_2O$, 0.001% $FeSO_4$. $7H_2O$	I	7	30	150	4 days	Colloidal chitin	----------
Streptomyces sp. NK1057 (33)	10 g/l chitin flakes, 0.5 $MgSO_4$, 0.2 KH_2PO_4, 0.3 $FeSO_4$, 0.3 Zn SO_4, 0.01 $MnCl_2$	I	7	40	150	5 days	Chitin flakes of crab shells	0.2% Inoculum size
Streptomyces griseus HUT 6037 (54)	0.2% colloidal chitin, 0.05% KCl ,0.1% K_2HPO_4, 0.05% $MgSO_4$ and 0.001% $FeSO_4$	I	7	30	ND	3 days	Colloidal chitin	----------
Streptomyces lydicus WYEC 108 (55)	Mineral salt solution supplemented with 1% colloidal chitin	I	7	25 – 30	250	5 days	Crab shell colloidal chitin	Spore concentration 2×10^5 / ml
Streptomyces thermoviolaceus OPC 520 (56)	1g yeast extract, 5 proteose, peptone, 5g colloidal chitin, 1g K_2HPO_4, 0.2 $MgSO_4$. $7H_2O$	I	7	50	ND	1 day	Colloidal chitin	----------
Aphanocladium album (57)	10g crystalline or colloidal chitin, 8.25g $(NH_4)_2$ SO_4, 0.5g $MgSO_4.7H_2O$, 0.5g KH_2PO_4, 0.1g Mn SO_4. H_2O, 0.058g Iron (III) citrate, 0.0015g nitrilotriacetate, 0.001g $CaCl_2$. $6H_2O$, 0.003g $ZnCO_4$. $7H_2O$, 0.0001g $CuSO_4.5H_2O$, 0.001g KCl $(SO_4)_2$. $12H_2O$, 0.0001g H_3 BO_3, 0.0001g H_3BO_3, 0.0001g Na_2 MoO_4. $2H_2O$ and 4,2g of 2 (n-morpholino) ethanesulfonic acid	I	5	28	80	208h ≈ 8 days (colloidal chitin) 340h ≈ 14 days (crystalline. chitin)	Crystalline or colloidal chitin	3 isoforms produced on crystalline chitin and 9 isoforms produced on colloidal chitin

Aspergillus sp. S1-13 (58)	0.5% colloidal chitin 0.3 glucose, 0.1% bactopepton, 0.14% $(NH_4)_2$ SO_4, 0.03% urea, 0.03% $MgSO_4.7H_2O$ and 0.1% (v/v) trace elements solution (0.5% $FeSO_4.$ $7H_2O$, 0.16% $MnSO_4.$ $2H_2O$, 0.14% Zn $SO_4.$ $7H_2O$ and 0.2% $CaCl_2$)	I	5	25	200	7 days	Colloidal chitin	Spore concentration 10^7 ml
Metarhizium anisopliae (59)	6g/l $NaNO_3$ + 7 chitin +20ml of salts solution (26g KCl, 26g Mg $SO_4.$ $7H_2O$, 76g KH_2PO_4 + 0.4 ml of trace elements solution (40mg Na_2 $B_4O_7.$ $7H_2O$, 400mg $CuSO_4.$ $5H_2O$, 10mg $FeSO_4$, 800mg $MnSO_4.$ $2H_2O$, 800mg Na_2 $MnO_4.$ H_2O 800mg $ZnSO_4.$ $7H_2O$	I	5	28	180	144 h = 6 days	Chitin (sigma)	Initial cell density 10^6 spores
Stachybotrys elegans (60)	Minimal synthetic medium (MSM) (0.5 % Mg $SO_4.$ $7H_2O$, 0.9 $K_2HPO_4.$ 0.2 KCl, 0.002 $FeSO_4.$ $7H_2O$, 0.002 $MnSO_4$, 0.002 Zn SO_4. The medium was supplemented with 1 mg /ml crab shell chitin and sodium nitrate $NaNO_3$	I	ND	24	115	3 days	Chitin (crab shell) sigma	Depending on the carbon source different isoforms of chitinase and β1.3 glucanase were produced
Trichoderma harzianum (29)	Wheat bran moistered (65.7%) with salt solution (0.5% (NH_4) NO_3, 0.2% KH_2PO_4, 0.1% NaCl, 0.1% $MgSO_4.$ $7H_2O$) + 1% colloidal chitin + 2% yeast extract	I	4.5	30	ND	4 days	Colloidal chitin	Spore concentration 4% 10^7 and initial moisture 65.7%
Verticillium lecanii F091(61)	4, 52% (w/v) maltoe, 1.79% marine peptone extract, 0.41% shrimp powder and 0.3% isolated soy protein.	I	4	24	150	6 days	Shrimp powder	Inoculum volume 2.5% and aeration rate 0.6 vvm.

Table 2: Chitinase production conditions and medium composition optimized using statistical approach

Microorganism/Reference	Pre-optimized medium and culture condition	Screened factors *	Optimized key factors**	Chitinase improvement value, rate or fold	Remarks
Pantoea dispersa (31)	chitin, 5.0; yeast extract, 0.5; (NH4)2SO4, 1.0; MgSO4·7H2O,0.3 and KH2PO4, 1.36 (g/l). pH 7.2 250 ml flasks and incubated for 144 h at 30±2 ◦C on (180 rpm).	Chitin, Glucose, Peptone, Yeast extract, Urea , (NH4)2SO4 ,NH4NO3 ,FeC6H5O7 , NaCl , MgCl2·6H2O , Na2SO4 3., CaCl2 , KCl , Na2CO3 ,KBr ,H3BO4 ,MgSO4·7H2O ,KNO3 ,andKH2PO4	chitin, peptone,yeast extract, urea,NH4NO3, NaCl, CaCl2, KBr, MgSO4·7H2O,KNO3 and KH2PO4 ,were significant and can be optimized for high chitinase production	4.21, 3.95, and 2.31-fold for chitinase,endochitinase and chitobiase , respectively.	-----
Stenotrophomonas maltophilia (62)	chitin (5 g/l), yeast extract (0.5 g/l), KH2PO4 (1.36 g/l), and MgSO4·7H2O (0.6 g/l)	Chitin, Maltose Galactose, Fructose, L-Asparagine Sodium Nitrate Yeast extract KH2PO4 MgSO4·7H2O	4.94 g/l chitin, 5.56 g/l maltose, 0.62 g/l,yeast extract, 1.33 g/l KH2PO4, and 0.65 g/l MgSO4·7H2O	--------	93.9% Model validity.
Alcaligenes	Chitin, 5.0; yeast extract, 0.5;	(NH4)2SO4 ,KH2PO4	Tween 20, yeast extract and	141%	-----

xylosoxydans IMI no. 385022 (30)	(NH4)2SO4, 1.0; MgSO4· 7H2O, 0.3 and KH2PO4, 1.36. pH 8.5	,MgSO4·7H2O , Tween 20 , Yeast extract , Chitin	chitin were found to be 0.12, 0.3 and 15 g/l,respectively.		
Chitinolyticbacter meiyuanensis SYBC-H1(63)	2.0 glucose, 4.0 peptone, 0.7 KH2PO4, 0.5 MgSO4·7H2O, 0.3 K2HPO4 and 0.02 FeSO4·7H2O g/l in 250 mL shake flasks and incubated at 30ºC for 12 hour on (200 rpm)	peptone, urea, corn steep liquor powder, ammonium sulfate, glucose, inulin, starch, sodium sulfate, fructose, and Magnesium sulfate	3.10 urea, 3.55 inulin, 3.80 chitin and 0.64 sodium sulfate(g/l)	15.5-fold	------
Enterobacter sp. NRG4 (64)	For all experimental designs, fermentations were carried out in 250-mL Erlenmeyer flasks, each having 4 g solid substrate. The constituents of the basal medium, i.e., KH2PO4 (0.1%) and MgSO4.7H2O (0.01%), were dissolved in distilled water and the pH was adjusted to 8.	Wheat bran/flake chitin ratio) Moisture level (%) Inoculum size (mL) Incubation time (h)	wheat bran-to-flake chitin ratio, 1 moisture level, 80%; inoculum size, 2.6 mL incubation time, 168 h	2.4-fold	-----
Serratia marcescens 97(65)	The important medium components identified by initial screening method of Plackett-Burman were colloidal chitin, yeast extract, MgSO4.7H2O and KH2PO4.	Peptone, Citric acid monohydrate, NaCl, MgSO4. 7H2O, (NH4)2 SO4 , Colloidal chitin , Yeast Extract ,KH2PO4	30ºC, initial pH of 6, inoculum size of 2.4 g/l and substrate concentration of 2. % for sugarcane bagasse,	predicted a chitinase activity of 38.637 U/ml	-----
Bacillus pumilus isolate U5 (12)	Powder chitin (2.5), yeast extract (0.25), (NH4)2SO4 (0.1), MgSO4.7H2O (0.05), CaCl2.2H2O (0.025), NaCl (0.5), KBr (0.05), MnCl2.4H2O (0.0005), ZnSO4.7H2O (0.0005), and FeSO4.7H2O (0.01), pH 6.5.	Powder chitin,Yeast extract (NH4)2SO4, MgSO4.7H2O CaCl2.2H2O, NaCl,KBr MnCl2.4H2O, ZnSO4.7H2O FeSO4.7H2O	chitin, yeast extract, MgSO4 and FeSO4 were found to be 4.76, 0.439, 0.0055 and 0.019 g/L, respectively	96.1 U/100 mL.	predicted response (97.67 U/100 mL)
Paenibacillus sp. D1. (32)	chitin, 5 ;0; yeast extract, 0,5; (NH4)2SO4, 1,0; MgSO4.7H2O, 0,3; and KH2PO4, 1,36 g/L, pH 7,2 at 30_C under shaking conditions (180 rev min)1) for 72 h.	Urea, K2HPO4, Tween 80, Chitin, MgSO4, FeCl3, Yeast extract	urea, 0,33; K2HPO4, 1,17; MgSO4, 0,3; yeast extract, 0,65 and chitin,3,75. g/L	2,56-fold	-----
Streptomyces sp. NK1057, NK528 and NK951(33)	Chitin medium containing g/l,crab shells chitin 10.0, MgSO4 0.5, K2HPO4 0.2, KH2PO4 0.3, FeSO4, ZnSO4 and MnCl2 0.01 pH 7.0 for strain NK1057 and NK528 and at pH 9.0 for NK951.The culture with 0.1 OD600 was used as inoculum (0.2%) at 40 ◦C for NK1057 and NK951 and at 30 ◦C for NK528 with 150 rpm.	Chitin, Yeast extract, Potassium hydrogen phosphate (dibasic) , Ammonium sulphate , Trace element solution , Inoculum density , pH , Temperature	yeast extract alone in all the three strains. ammonium sulphate and pH for Streptomyces sp. NK528 and NK951 whereas chitin and yeast extract for Streptomyces sp. NK1057	29, 9.3 and 28%, for Streptomyces sp. NK1057, NK528 and NK951 respectively.	the error was not more than 11%
Streptomyces sp. DA11(34)	1 g/L (NH4)2SO4, 10 g/L powder chitin, 1 g/L PO4 3_ (KH2PO4 0.3 g/L, K2HPO4 0.7 g/L), MgSO4_7H2O 0.5 g/L, FeSO4 0.01 g/L and ZnSO4_7H2O 0.01 g/L, pH 7.0. Media were prepared with artificial sea water (ASW) and. flask (250 mL) with 100 mL of fermentation medium on (180 rpm) at 28 8C.	Galactose, Peptone, PO43, Powder chitin, Colloidal chitin MgSO4_7H2O, FeSO4 , ZnSO4_7H2O	galactose, colloidal chitin and MgSO4_7H2O, were found to be 5.00 g/L, 2.62 g/L and 0.10 g/L,respectively.	39.2-fold	-----
Basidiobolous ranarum (35)	(N.A.D)	(N.A.D)	1.5% colloidal chitin, 0.125% lactose, 0.025% malt extract and 0.075% peptone.	7.71 fold.	
Aspergillus terreus(36)	fish scales waste, 20 g/L; (NH4)2SO4, 2; K2HPO4, 1; MgSO4.7H2O, 0.5; KCl, 0.5; NaCl, 5; CaCl2, 0.02; FeSO4.7H2O, traces and pH 6 and dispensed in 250 ml Erlenmyer flasks. Standard inocula(14.6 x 106spores/ml) were used to inoculate the flasks which were then incubated at 30 ± 2°C at 150 rpm for 5 days	Eleven culture parameters SH, fish scales waste; GL, glucose; PE, peptone; NH, (NH4)2SO4; UR, urea; MG, MgSO4.7H2O; FE, FeSO4.7H2O; MN, MnSO4.2H2O; ZN, ZnSO4.7H2O; CO, CoCl2; SP, spore number.	FeSO4.7H2O (9.5), glucose (6.5) and MnSO4.2H2O (4.7) (g/l)	1.81 folds	99% model validity.
Alternaria alternata (66)	Glucose, 0.3; peptone, 0.1; (NH4)2SO4, 0.14; urea, 0.03; MgSO4.7H2O,	SH, shrimp shellfish; GL, glucose; PE, peptone; NH, (NH4)2SO4; UR,	glucose, 9; MnSO4.2H2O, 3.2 and CoCl2, 2g/l	1.8 folds	99.68% Model validity.

	0.03; FeSO4.7H2O, 0.5; MnSO4.2H2O, 0.16; ZnSO4.7H2O, 0.14; CoCl2, 0.2, shrimp shellfish waste,2 g/l	urea; MG, MgSO4.7H2O; FE, FeSO4.7H2O; MN, MnSO4.2H2O; ZN, ZnSO4.7H2O; CO, CoCl2; SP, spore number.			
Lecanicillium muscarium CCFEE 5003(67)	basal fermentation medium (BM): yeast nitrogen base (YNB) 1%, colloidal chitin 1% Silicone antifoam 0,2% was added	(NM)	Agitation and aeration are **327** rpm and 1.1 vvm, respectively	23%	The bioreactor used was 2-l (total volume) bench-top stirred tank reactor (STR)
Trichoderma harzianum792 (68)	(NM)	Peptone, (NH4)2 SO4, NaH2PO4, KH2PO4, MgSO4. 7H2O, Citric acid monohydrate, Urea, Malt extract	substrate concentration 1.8g/l of rice bran, initial pH 6.5, fermentation temperature 33°C and inoculum size 2.4%.	A maximum chitinase yield of 66.5U/ml	-----

*: using Plackett-Burman Central composite design (CCD) experimental design; **: optimized by Box-Behnken factorial design of Response Surface Methodology (RSM); NM: not mentioned.; (N.A.D): not available data

Conclusion:

The optimization of medium composition and production conditions plays a crucial role in the microbial production of chitinases and help to understand the physiology of enzyme production, recognizing the key factors, setting the best bioprocess (fermentation) conditions which pave the way for the industrial scale-up with low cost and high enzymatic yield.

ACKNOWLEDGEMENT

Thanks are due to Dr.Safi, K. for the helpful comments on earlier versions of the paper and the assistance in handling the bibliography.

REFERENCES

[1] Zargar, V., M. Asghari and A. Dashti, 2015. A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives, and Applications. Chem.Bio.Eng. Reviews, 2: 204-226.
[2] Jung, B.O., S. Roseman and J.K. Park, 2008. The central concept for chitin catabolic cascade in marine bacterium *Vibrios.* Macromolecular research, 16: 1-5.
[3] Sharma, N., K.P. Sharma, R.K. Gaur and V.K. Gupta, 2011. Role of chitinase in plant defense. Asian Journal of Biochemistry, 6: 29-37.
[4] Flach, J., P.E. Pilet and P. Jolles, 1992. Whats new in chitinase research? Experientia, 48: 701-716.
[5] Patil, R.S., V. Ghomade and M.V. Deshpande, 2000. Chitinolytic enzymes: an exploration. Enzyme Microbial Technology, 26: 473-483.
[6] Herrera-Estrella, A. and I. Chet, 1999. Chitinases in biological control. EXS. 87: 171-84.
[7] Kzhyshkowska, J., S. Yin, T. Liu, V. Riabov, I. Mitrofanova, 2016. Role of chitinase-like proteins in cancer. Biol Chem., 397(3): 231-47.
[8] Kastrup, J., 2012. Can YKL-40 be a new inflammatory biomarker in cardiovascular disease? Immunobiology, 217(5): 483-491.
[9] Shanmugaiah, V., N. Mathivanan, N. Balasubramanian and P.T. Manoharan, 2008. Optimization of cultural conditions for production of chitinase by *Bacillus laterosporous* MML2270 isolated from rice rhizosphere soil. African Journal of Biotechnology, 7(15): 2562-2568.
[10] Jami Al Ahmadi, K., M. Tabatabaei Yazdi, M. Fathi Najafi, A.R. Shahverdi, M.A. Faramarzi, G. Zarrini and J. Behravan, 2008. Optimization of medium and cultivation conditions for chitinase production by the newly isolated: *Aeromonas sp*. Biotechnology, 7: 266-272.
[11] Abd-Aziz, S., C.C. Fernandez, M.M. Salleh, R.M. Illias and M.A. Hassan, 2008. Effect of agitation and aeration rates on chitinase production using *Trichoderma virens* UKM1 in 2-l stirred tank reactor. Appl Biochem Biotechnol., 150: 193-204.
[12] Tasharrofia, N.B., S. Adrangie, M. Fazelia, H. Rastegarc, M.R. Khoshayandd and M.A. Faramarzia, 2011. Optimization of chitinase production by *Bacillus pumilus* using Plackett-Burman design and response surface methodology. Iranian Journal of Pharmaceutical Research, 10(4): 759-768.

[13] Cheba, B., T.I. Zaghloul, A.R. EL-Mahdy and M.H. EL-Massry, 2011. Enhanced production of *Bacillus sp*. R2 chitinase through cell immobilization. ACT-Biotechnology Research Communications., 1(1): 8-13.
[14] Escott, G. and D.J. Adams, 1995. Chitinase activity in human serum and leukocytes, Infec. Imnun., 63(12): 4770-4773.
[15] Matsumoto, K.S., 2006. Fungal chitinases. Advances in Agricultural and Food Biotechnology, 6: 289-304.
[16] Rattanakit, N., A. Plikomol, S. Yano, M. Wakayama and T. Tachiki, 2002. Utilization of shrimp shell fish waste as a substrate for solid-state cultivation of *Aspergillus* sp. S1-13: Evaluation of a culture based on chitinase formation which is necessary for chitin assimilation. J. Biosc. Bioeng., 93(6): 550-556.
[17] Hart, L., S. Zach and V. Seid-Seiboth, 2011. Review Fungal chitinases: diversity, mechanistic properties and biotechnological potential. Appl Microbiol Biotechnol., 93(2): 533-43.
[18] Gummadi, S.N., D. Kumar, S.D. Swati and S.S. Kumar, 2009. Industrially Important Carbohydrate Degrading Enzymes from Yeasts: Pectinases, Chitinases, and β-1,3-Glucanases. 673-691, chapter III, in Yeast Biotechnology: Diversity and Applications, Springer Science, XXII, 744.
[19] Dünkler, A., A. Walther, C.A. Specht and J. Wendland, 2005. *Candida albicans* CHT3 encodes the functional homolog of the Cts1 chitinase of Saccharomyces cerevisiae. Fungal Genet Biol., 42(11): 935-947.
[20] Bhattacharya, D., A. Nagpure and R.K. Gupta, 2007. Bacterial chitinases: properties and potential. Crit Rev Biotechnol., 27(1): 21-8.
[21] Itoi, S., Y. Kanomata, S. Uchida, K. Kadokura, T. Nishio, T. Oku and H. Sugita, 2012. Effect of the C-terminal domain of *Vibrio proteolyticus* chitinase A on the chitinolytic activity in association with pH changes. Letters in Applied Microbiology, 54: 441-446.
[22] Singh, N.A. and V. Shanmugam, 2012. Cloning and characterization of a bifunctional glycosyl hydrolase from an antagonistic *Pseudomonas putida strain* P3(4). J. Basic Microbiol., 52: 340-349.
[23] Watanabe, T., W. Oyanagi, K. Suzuki and H. Tanaka, 1990. Chitinase system of *Bacillus circulans* WL-12 and importance of chitinase Al in chitin degradation. J. Bacteriol., 172(7): 4017-4022.
[24] Takayanagi, T., K. Ajisaka, Y. Takiguchi and K. Shimahara, 1991. Isolation and characterization of thermostable chitinases from *Bacillus licheniformis* X-7u. Biochimica, Biophsica Acta., 1378: 404-410.
[25] Ohishi, K., M. Yamagishi, T. Ohta, M. Suziki, H. Izumida, H. Sano et al., 1996. Purification and properties of two chitinases from *Vibrio alginolyticus* H-8. J. Ferment. Bioeng., 82: 598-600.
[26] Aunpad, R., and W. Panbamgrad, 2003. Cloning and characterization of the constitutively expressed chitinase C gene from a marine bacterium *Salinivibrio costicola* strain 5SM-1. J. Biosc. Bioeng., 96: 529-536.
[27] Irfan, M., M. Nadeem, S. Quratulain, 2014. One-factor-at-a-time (OFAT) optimization of xylanase production from *Trichoderma viride*-IR05 in solid-state fermentation; Journal of Radiation Research and Applied Sciences, 7: 317-326.
[28] Strobel, R.J., G.R. Sullivan, 1999. Experimental design for improvement of fermentations. In: Demain AL, Davies JE, editors. Manual of industrial microbiology and biotechnology. 2nd ed. Washington:American Society for Microbiology, pp: 80-93.
[29] Felse, P.A., T. Panda, 1999. Self-directing optimization of parameters for extracellular chitinase production by *Trichoderma harzianum* in batch mode. Proc Biochem, 34: 563-6.
[30] Vaidya, R., P. Vyas and H.S. Chhatpar, 2003. Statistical optimization of medium components for the production of chitinase by *Alcaligenes xylosoxydans*. Enzyme and Microbial Technology, 33: 92-96.
[31] Gohel, V., T. Chaudhary, P. Vyas and H.S. Chhatpar, 2006. Statistical screenings of medium components for the production of chitinase by the marine isolate *Pantoea dispersa* Biochemical Engineering Journal, 28: 50-56.
[32] Singh, A.K., G. Mehta and H.S. Chhatpar, 2009. Optimization of medium constituents for improved chitinase production by *Paenibacillus sp.* D1 using statistical approach. Letters in Applied Microbiology, 49: 708-714.
[33] Nawani, N.N. and B.P. Kapadnis, 2005. Optimization of chitinase production using statistics based experimental designs.Process Biochemistry, 40: 651-660.
[34] Yue Han, Zhiyong, Li, Xiaoling Miao and Fengli Zhang, 2008. Statistical optimization of medium components to improve the chitinase activity of Streptomyces sp. Da11 associated with the South China Sea sponge *Craniella australiensis*. Process Biochemistry, 43: 1088-1093.
[35] Mishra, P., P.R. Kshirsagar, S.S. Nilegaonkar and S.K. Singh, 2012. Statistical optimization of medium components for production of extracellular chitinase by *Basidiobolus ranarum*: a novel biocontrol agent against plant pathogenic fungi, Journal of Basic Microbiology, 52(5): 539-48.
[36] Ghanem, K.M., S.M. Al-Garni and N.H. Al-Makishah, 2010. Statistical optimization of cultural conditions for chitinase production from fish scales waste by *Aspergillus terreus*.African Journal of Biotechnology, 9(32): 5135-5146.
[37] Shimosaka, M., M. Nogawa, X. Wang, M. Kumehara and M. Okazaki, 1995. Production of two

chitosanases from a chitosan-assimilating bacterium, *Acinetobacter sp*. strain CHB101. App. Environ. Microbiol., 61(2): 438-442.
[38] Guo, S.H., J.X. Chen and W.C. Lee, 2004. Purification and characterization of extracellular chitinase from *Aeromonas Schubertii*. Enz. Microb. Technol., 35: 550-556.
[39] Tsujibo, H., Y. Yoshida, K. Miyamoto, C. Imada, Y. Okami and Y. Inamori, 1992. Purification, properties and partial amino acid sequence of chitinase from a marine *Alteromonas* sp. strain 07. Can. J. Microbiol., 64: 472-478.
[40] Wang, S.Y., A.L. Moyne, G. Thottappilly, S.J. Wu, R.D. Locy and N.K. Singh, 2001. Purification and characterization of *Bacillus cereus* exochitinase. Enz. Microb. Technol., 28: 492-498.
[41] Frandberg, E., and J. Schnurer, 1994. Chitinolytic properties of *Bacillus pabuli* k1. J. Appl. Bacteriol, 76: 361-367.
[42] Bhushan, B and G.S. Hoondal, 1998. Isolation, purification and properties of a thermostable chitinase from an alkalophilic *Bacillus sp*. BG-11. Biotechnol. Lett., 20(2): 157-159.
[43] Kim, P., T. Kang, K. Chung, I. Kim and K. Chung, 2004. Purification of a constitutive chitosanase produced by Bacillus sp. MET 1299 with cloning and expression of the gene. FEMS.Microbiol. Lett., 240: 31-39
[44] Wen, C., C. Teseng, C. Cheng and Y. Li, 2002. Purification, characterization and cloning of a chitinase from *Bacillus sp.* NTU2. Biotechnol. App. Biochem., 35: 213-219.
[45] Sakai, K., A. Yokota, H. Kurokawa, M. Wakayama and M. Moriguchi, 1998. Purification and characterization of three thermostable endochitinases of a noble *Bacillus strain*, MH-1 isolated from chitin-containing compost. App. Environ. Microbiol., 64(9): 3397-3402.
[46] Yuli, P.E., M.T. Suhartono, Y. Rukayadi, J.K. Hwanj and Y.R. Pyun, 2004. Characterisic of thermostable chitinase enzymes from the Indonesian *Bacillus sp*. 13.26. Enz. Microb. Technol., 35: 147-153.
[47] Sakai, K., M. Narihara, Y. Kasama, M. Wakayama and M. Moriguchi, 1994. Purification and characterization of thermostable β-N-acethyl hexosaminidase of *Bacillus stearothermophilus* CH-4 isolated from chitin-containing compost. Appl. Environ. Microbiol., 60(8): 2911-2915.
[48] Chen, H.C., M.F. Hsu and S.T. Jiang, 1997. Purification and characterization of an exo-N, N-diacetylchitobiohydrolase - like enzyme from *Cellulomonas flavigena* NTOU1. Enz.Microb. Technol., 20: 191-197.
[49] Dahiya, N., R. Tewari, R.P. Tewari and G.S. Hoondal, 2005. Chitinase from *Enterobacter* sp. NRG4: its purification, characterization and reaction pattern. Electronic. J. Boitechnol., 8(2): 134-145.
[50] Wang, S.L and W.T. Chang, 1997. Purification and characterization of two bifunctional chitinase/lysozymes extracellularly produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium. App. Environ. Microbiol., 63(2): 380-386.
[51] Suginta, W., A. Vongswnan, C. Songsiritthigul, H. Prinz, P. Estibeiro, R.R. Duncan, J. Svasti, F. Linda and Gilmore, 2004. Endochitinase from *Vibrio carchariae*: cloning, expression mass and sequence analysis, and chitin hydrolysis. Arch. Biochem. Biophys., 424: 171-180.
[52] Yamaoka, H., H. Hayashi, S. Karita, T. Kimura, K. Sakka and K. Ohmiya, 1999. Purification and some properties of chitinase from *Xanthomonas sp*. strain AK. J. Biosc. Bioeng., 88(3): 328-330.
[53] El-sayed, E., S.M. Ezzat, M.F. Ghaly, M. Mansour and M.A. El-Bohey, 1999. Purification and characterization of two chitinases from *Streptomyces albovinaceus* S-22. J. Union Arb. Biol. 8 (B). Microbiology and viruses 149-163, 6th international conference.
[54] Tanabe, T., T. Kawase, T. Watanabe, Y. Uchida and M. Mitsutomi, 2000. Purification and characterization of a 49 – Kda chitinase from *Streptomyces griseus* HUT 6037. J. Biosc. Bioeng., 89(1): 27-32.
[55] Mahadevan, B and D.L. Crawford, 1997. Properties of the chintinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC 108. Enz. Microb. Technol., 20: 489-493.
[56] Tsujibo, H., K. Minoura, K. Miyamoto, H. Endo, M. Moriwaki and Y. Inamori, 1993. Purification and properties of a thermostable chitinase from *Streptomyces thermoviolaceus* OPC-520. Appl. Environ. Microbiol., 59(2): 620-622.
[57] Blaiseau, P.L., C. Kunz, R. Grison, Y. Bertheau and Y. Brygoo, 1992. Cloning and characterization of chitinase gene from the hyper parasitic fungus *Aphanocladium album*. Curr. Genet., 21: 61-66.
[58] Rattanakit, N., A. Plikomol, S. Yano, M. Wakayama and T. Tachiki, 2002. Utilization of shrimp shell fish waste as a substrate for solid-state cultivation of *Aspergillus sp*. S1-13: Evaluation of a culture based on chitinase formation which is necessary for chitin assimilation. J. Biosc. Bioeng., 93(6): 550-556.
[59] Pinto, A.D., C.C. Barreto, A. Schrank, C.J. Ulhoa and M.H. Vainstein, 1997. Purification and characterization of an extracellular chitinase from the entomopathogen *Metarhizium anisopliae*. Can. J. Microbiol., 43: 322-327.
[60] Tweddell, R.J., S.H. Jabaji-Hare, P.M. Charest, 1994. Production of chitinase and β-1, 3- glucanase by *Stachybotrys elegans*, a mycoparasite of *Rhizoctonia solani*. Appl. Environ. Microbiol., 60: 489-498.
[61] Liu, B.L., P.M. Kao, Y.M. Tzeng and K.C. Feng, 2003. Production of chitinase from *Verticillium lecanii.*

Enz. Microb. Technol., 33: 110-115.
[62] Khan Minhaj, A., H. Rifat, A. Mahboob, M.Z. Abdin and S. Javed, 2010, Optimization of culture media for enhanced chitinase production from a novel strain of *Stenotrophomonas maltophilia* using response surface methodology.J. Microbiol. Biotechnol., 20(11): 1597-1602.
[63] Hao, Z., Y. Cai, X. Liao, X. Zhang, Z. Fang and Z. Dabing, 2012.Optimization of nutrition factors on chitinase production from a newly isolated *Chitiolyticbacter meiyuanensis* SYBC-H1. Braz. J. Microbiol., 43 (1): 177-186.
[64] Dahiya, N., R. Tewari, P. Tiwari, Ram and G.S. Hoondal, 2005.Chitinase production in solid-state fermentation by *Enterobacter sp*.NRG4 using statistical experimental design, Current Microbiol., 51: 222-228.
[65] Sudhakar, P., and P. Nagarajan, 2011. Production of chitinase by solid state fermentation from *Serratia marcescens*. International Journal of Chem.Tech. Research, 3(2): 590-598.
[66] Ghanem, K.M., F.A. Al-Fassi and R.M. Farsi, 2011. Statistical optimization of cultural conditions for chitinase production from shrimp shellfish waste by *Alternaria alternate*.African Journal of Microbiology Research, 5(13): 1649-1659.
[67] Fenice, M., P. Barghini, L. Selbmann and F. Federici, 2012. Combined effects of agitation and aeration on the chitinolytic enzymes production by the Antarctic fungus *Lecanicillium muscarium* CCFEE 5003. Microb. Cell Fact., 11: 12.
[68] Sudhakar, P. and P. Nagarajan, 2011. Process optimization for chitinase production by *Trichoderma harzianum.* As. J. Food Ag-Ind., 4(2): 91-102.