THE SCIENCE OF BEVERAGES
VOLUME 2

# PROCESSING AND SUSTAINABILITY OF BEVERAGES

Edited by
Alexandru Mihai Grumezescu
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# PECTINASES: PRODUCTION AND APPLICATIONS FOR FRUIT JUICE BEVERAGES

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## 8.1 Introduction

Pectin is a complex polysaccharide and presents prominently in the middle lamella of plant cell wall (Kertesz, 1951). Pectin forms about one-third of the cell wall dry substances of dicotyledonous plants (Jarvis et al., 1988). The pectic substances play an important role in cell adhesion and provide mechanical strength to the cell wall (Jarvis, 1984). Pectic substances are acidic heteropolysaccharides with molecular masses ranging from 23 000 to 360 000 D (Newbold and Joslyn, 1952). They occur in varying amounts in all higher plant tissues. Fruits and vegetables contain varying amount of pectin from 0.2% to 0.5% in tomatoes to 30% to 35% in citrus peel (Tapre and Jain, 2014). The presence of pectin is crucial to the formation of fruit products from clear juices to jams, jellies, and marmalades. The first information on water-soluble jellying substances in fruits was published by Vauquelin (1790). In 1825, Braconnot showed de-esterification of pectin to pectic acid and 162 years later, Vennigerholz and Wales (1987) showed that digestion of the tissues with pectolytic enzymes leads to dissolution of the middle lamella and thereby cell separation.

Pectic enzymes have been used as bulk heterogenous preparations by fruit processing industries. Mostly these industries use fungal enzymes supplied by Gist-Brocades, Novo-Nordisk, Biocon, A.T.P, ABM Sturge, Genencore, Amano, and Shin-Nihon (Pilnik and Voragen, 1993). However, relatively pure preparations of various immobilized pectic enzymes have also been employed in the form of bioreactor for industrial applications (Nighojkar et al., 1995).

This chapter deals with the occurrence, classification, structure, composition and degree of esterification of pectin, commercial pectin

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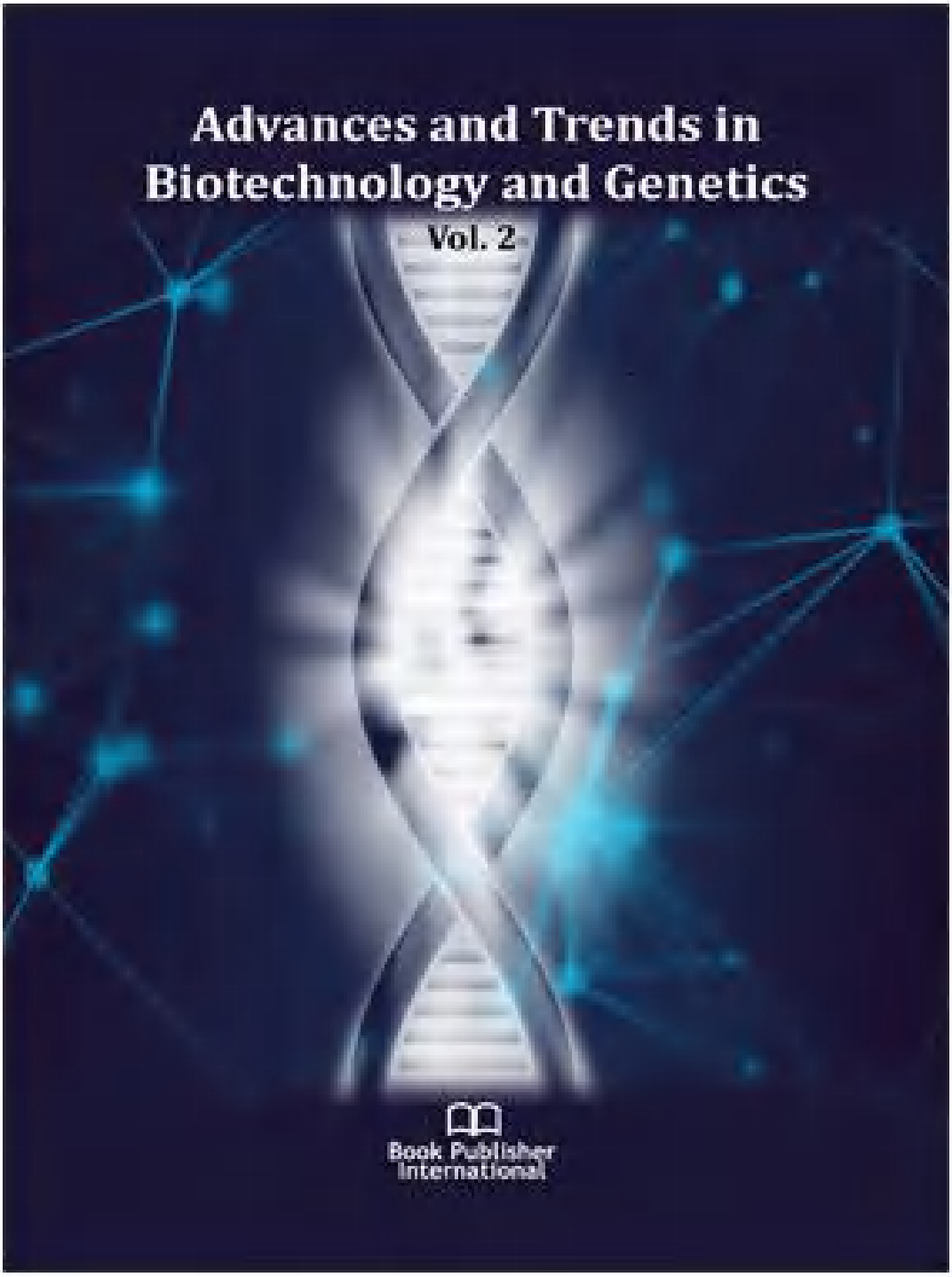
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## Synopsis

*This book covers all areas of biotechnology, genetics and other related fields. The contributions by the authors include pectin methylesterase, ion exchange chromatography, gel filtration chromatography, solid state fermentation, Streptomyces, enzyme, renal-toxicity, nicotine, endo-1,4-β-xylanase, birchwood xylan, animal feedstocks, xylooligosaccharides, vitamin, chronic exposure, microarrays, RNA-seq, Background Subtraction (BS), Differentially Expressed Genes (DEGs), diabetes mellitus, β-cell of pancreas, stem cell therapy, pluripotent stem cells, hyperthermophilic, alkaline, methaemoglobin, carboxyhaemoglobin, spectrophotometer, pregnant women and hepatitis B, malaria, artesunate-amodiaquine, artemether-lumefantrine, pfK13-propeller, PfK13A369G, Cold Shock Proteins (CSP), Cold Inducible RNA-binding Protein (CIRP), RNA Binding Motif 3 (RBM3) etc. This book contains various materials suitable for students, researchers and academicians in the field of biotechnology and genetics.*


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# Purification and Characterization of Pectin Methylesterase Produced in Solid State Fermentation by Aspergillus tubingensis | Chapter 03 | Advances and Trends in Biotechnology and Genetics Vol. 2

*Aim:* *Purification and characterization of pectin methylesterase produced by Aspergillus tubingensis in solid state fermentation.*

*Study Design:* *Pectin methylesterase enzyme produced by A. tubingensis was extracted from the fermented solid medium and purified using chromatographic techniques. The purified enzyme was characterized for physico-chemical and kinetic properties.*

*Place and Duration of Study:* *Experiments were performed at the School of Biotechnology, Devi Ahilya University, Indore, INDIA and Maharaja Ranjit Singh College of Professional Sciences, Indore, INDIA, between October, 2014 and August, 2015.*

*Methodology:* *The enzyme was extracted and purified using ammonium sulphate fractionation, ion exchange chromatography (IEC) using CM- cellulose and gel filtration chromatography (GFC) using Sephadex G-100. The molecular weight of the purified enzyme was determined using native polyacrylamide gel electrophoresis (Native PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). The purified enzyme was characterized to determine the pH and temperature optima. Thermostability, pH stability and substrate kinetics were studied for purified pectin methylesterase.*

*Results:* *The acidic pectin methylesterase of Aspergillus tubingensis was purified to 20.3 fold with a 47.7% recovery through IEC on CM- cellulose and GFC using Sephadex G-100. The purified enzyme had a specific activity, 112.6 U/mg. The SDS-PAGE revealed that the enzyme was monomeric with a molecular weight of 45.7 kDa. The optimum pH and temperature were 4.6 and 50°C, respectively. This enzyme was stable over a wide pH range (3.0–8.0) and at relatively high temperature at 50°C for 1 h. The Km and Vmax values of pectin methylesterase towards citrus pectin were 33.3 mg/l and 251.2 µmol/ml/min, respectively. In addition, the enzyme activity increased by about 16% in the presence of 5 mM $Mg^{2+}$.*

*Conclusion:* *The pectin methylesterase enzyme of A. tubingensis has been purified up to homogeneity and found to be monomeric on SDS-PAGE. Enzyme characterization revealed that purified enzyme worked optimally in acidic conditions and was stable at wider pH range.*

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**8th & 9th December-2019**

**Focal Theme**

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IYSC-2018

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8th & 9th May 2018

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*Global Research: Energy, Ethics and Enthusiasm*

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*Widespread Research: Strengthening Nations and Spreading Happiness*

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and

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8th & 9th December-2017

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**IYSC-2017**

*Interdiciplinary research: contribution towards global strength*


8th & 9th May 2016

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*Digitization in Research for Cultural, Commercial & Scientific Development*



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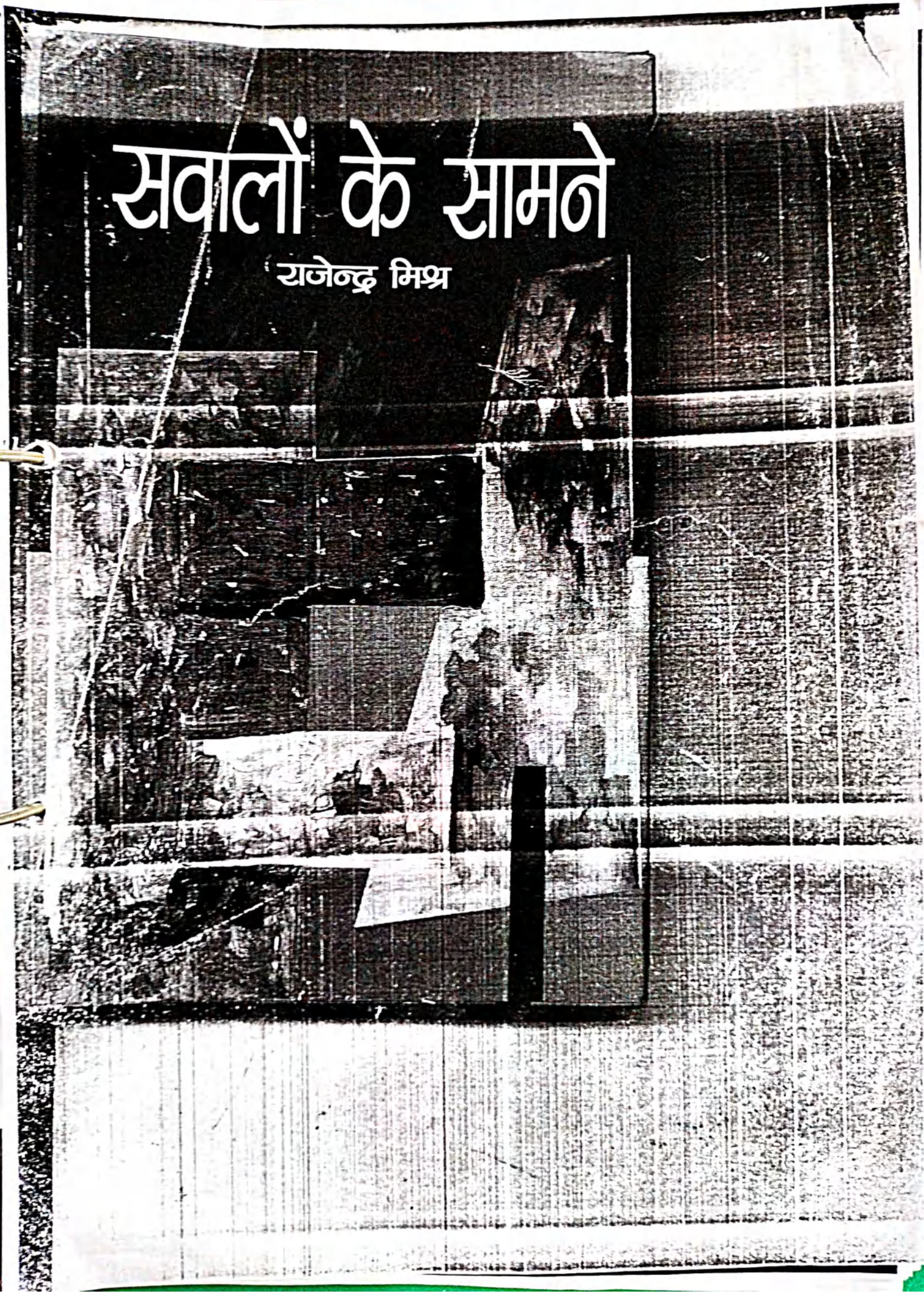


# वीडियो साक्षात्कार

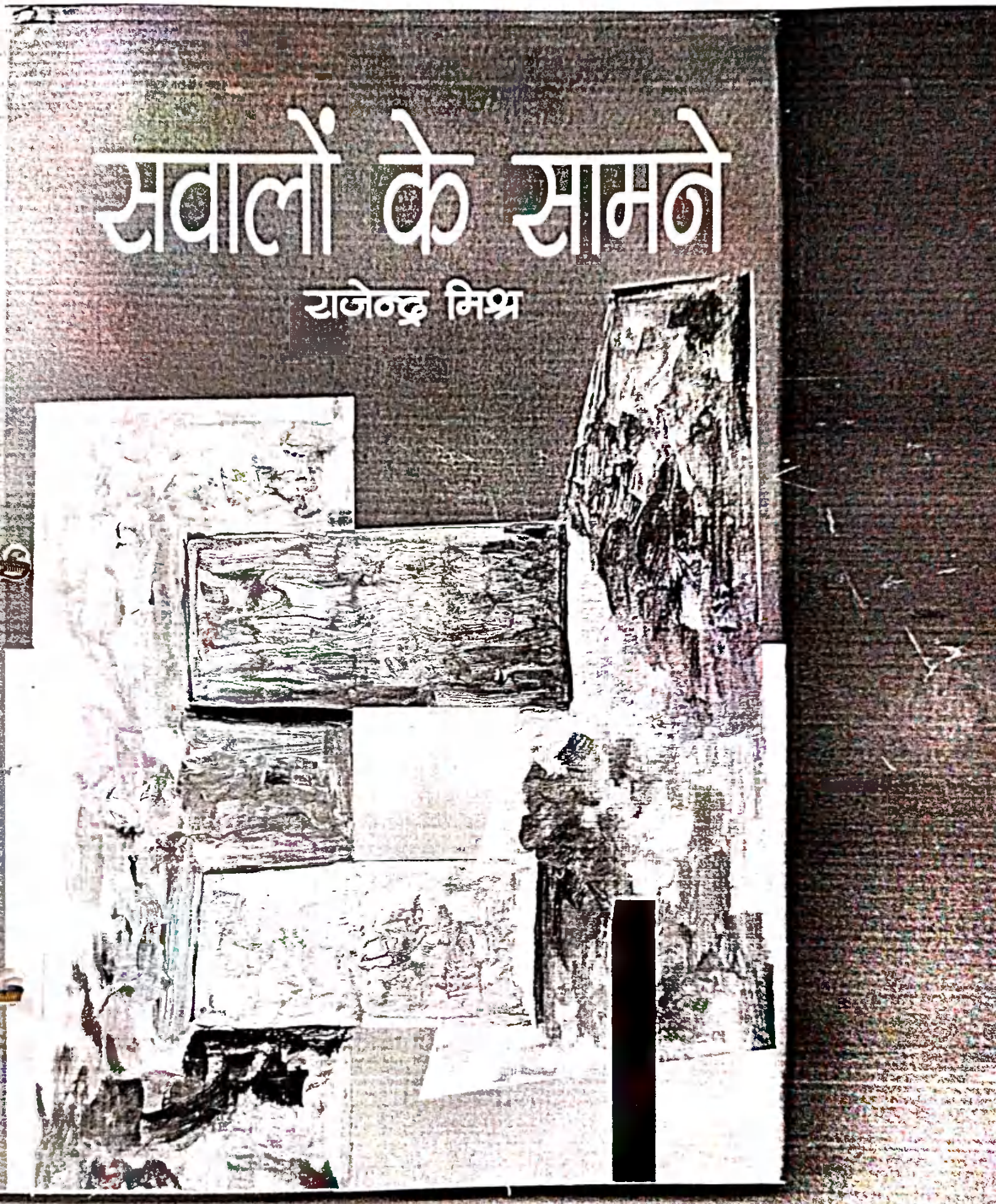
## डॉ॰ पुष्पेंद्र दुबे

1. हम अपने पाठकों को बताना चाहेंगे कि आज हमारे साथ में हैं, डॉ॰ राजेंद्र मिश्र, वह इंदौर के ही नहीं वरन अपने देश की एक साहित्यिक विभूति के रूप में जाने जाते हैं। हाल ही में आपके समग्र साहित्य की रचनावली ग्यारह खंडों में प्रकाशित होकर आई हैं। यह इंदौर के लिए बड़े सौभाग्य की बात है कि वे एकमात्र ऐसे साहित्यकार हैं, जिनकी प्रकाशित सामग्रियों का रचनावली के रूप में प्रकाशन हुआ है। आप इंदौर विश्वविद्यालय के गोल्ड मेडलिस्ट रहे हैं। साथ में आपने डी॰लिट्॰ की उपाधि भी हासिल की और हमारे बीच में आज वे उपस्थित हैं। हम उनसे यह जानना चाहेंगे कि उनकी रचना- प्रक्रिया, उनकी रचनाधर्मिता किस प्रकार से रही। डॉ॰ राजेंद्र मिश्र जी ने हिंदी साहित्य की लगभग सभी विधाओं में अनवरत रूप से अपनी लेखनी चलाई है और हिंदी साहित्य को अनुपम उपलब्धियों से सँवारा है। उन्होंने अपने साहित्य के माध्यम से अनेक नई स्थापनाएँ की हैं। तो आइए हम उनसे जानकारी लेते हैं कि आखिर उनको सृजन करने के लिए प्रेरणा कहाँ से प्राप्त हुई, और वे क्या सोचते हैं अपने समग्र साहित्य के बारे में?

हर साहित्यकार को, विशेषकर मुझ जैसे साहित्यकार को सृजन की प्रेरणा अपने भीतर से ही मिलती है। भीतर से जो प्रेरणा होती है, वह समय के साथ व्यक्ति के अस्तित्व को जोड़ती है। मैंने इसीलिए जो भी लिखा है, उसका आरंभ ही समय से हुआ है। मेरा पहला कविता-संकलन 'अपने समय में' इस बात प्रतीक है। उसमें मैंने लिखा है कि मैं टाइपराटर पर जब बैठ जाता हूँ तो मेरी कविताएँ जन्म लेने लगती हैं। यह बात कुछ अजीब-सी लगेगी, लेकिन मशीन के इस युग में इस बात को हम स्वीकार कर सकते हैं। अपने समय ने मुझे बहुत प्रभावित किया है। जब आपातकाल का आरंभ हुआ, उस समय मैं बेचैन था। अपनी स्वतंत्रता के लिए क्योंकि मैं मानता हूँ अगर रचनाकार स्वतंत्र नहीं है तो वह सृजन कर ही नहीं सकता। इसीलिए 'अपने समय में नहीं' की मेरी सारी कविताएँ

# सवालों के सामने

राजेन्द्र मिश्र



"My theory of 'Trusteeship' is no makeshift, certainly no camouflage. I am confident that it will survive all other theories. It has the sanction of philosophy and religion behind it... No other theory is compatible with nonviolence."


Siby K. Joseph
Bharat Mahodaya
Ram Chandra Pradhan

Editors


# Trusteeship

## A Path Less Travelled

अंत में इस बात की ओर भी ध्यान आकृष्ट करना होगा की धन या मुद्रा ही पूँजी नहीं है, श्रम भी पूँजी है। किन्तु आज श्रम के गौरव की प्रतिष्ठा नहीं है। श्रम के औचित्य को देखे तो ट्रस्टीशिप का सिद्धान्त धनपतियों पर पहले लागू होना चाहिए, श्रमिकों पर बाद में।

गांधीवादी अर्थव्यवस्था श्रम और पूँजी के बीच तथा समाज के विभिन्न वर्गो के बीच समन्वय स्थापित करके संघर्ष समाप्ति और शांति स्थापना करने में सक्षम है। आज विश्व को इसकी जरुरत है।

## संदर्भ

1. *हरिजन,* 25-10-1952.
2. Nirmal Kumar Bose, *Selections from Gandhi*, (Ahmedabad: Navajivan, 1996), p. 90.

Pyarelal Nayyar

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# भविष्य का समाज और ट्रस्टीशिप: सिद्धांत और व्यवहार

पुष्पेंद्र दुबे

### वैश्विक परिस्थिति और भारत

इक्कीसवीं शताब्दी में भारत में नयी संभावनाएं दिखाई दे रहीं हैं। विश्व में राजनैतिक साम्राज्यवाद समाप्त हो गया है, साम्यवादी विश्व बिखर रहा है, लोकतंत्र का तेजी से प्रसार हो रहा है, संचार क्रांति, सूचना क्रांति ने पूरे विश्व को एक गांव में रूपांतरित कर दिया है। आर्थिक क्षेत्र में विश्व अर्थव्यवस्था ने एक निश्चित आकार ले लिया है। शिक्षा के लोकव्यापीकरण और ज्ञान आधारित समाज के विस्तार ने नवीन वैश्विक मानव मूल्यों को अंगीकार किया है। वस्तुतः नये विश्व और नये मनुष्य की ही रचना हो रही है। शोषण और हिंसा पर आधारित समाज में से शोषण मुक्त और अहिंसक समाज का जन्म कैसे हो, इस विषय पर बीसवीं सदी के संत और मनीषी आचार्य विनोबा भावे के विचार अत्यंत प्रासंगिक हैं।



बीसवीं शताब्दी के पूर्वार्द्ध में दुनिया के लगभग 150 देश गुलाम थे और भारत गुलाम देशों में सबसे बड़ा देश था। महात्मा गांधी के नेतृत्व में भारत ने आजादी की अनूठी लड़ाई लड़ी और वह स्वतंत्र हुआ। भारत के आजाद होने के बाद दुनिया के दूसरे गुलाम देश भी आजाद हुए। लेकिन दूसरी ओर दुनिया के देश उपभोक्तावादी संस्कृति, औद्योगिक संस्कृति की आर्थिक – सामाजिक – मानसिक – नैतिक गुलामी में फंस गये हैं।

# साम्ययोगी समाज के मौलिक चिंतक-विनोबा

डॉ. पुष्पेंद्र दुबे

विनोबा विचार का जितना अध्ययन, मनन, चिंतन और थोड़ा बहुत कार्य रूप में परिणत करते जाते हैं, उतना विश्वास होता जाता है कि मनुष्य जीवन की उन्नति का इससे सरल मार्ग और क्या हो सकता है। यद्यपि हम इस सरल मार्ग पर कई बार गिरते–पड़ते रहते हैं। तब स्वामी श्रद्धानंद द्वारा अपने शिष्यों को कही एक बात हमेशा स्मरण हो आती है 'इस संसार की अंधियारी में किसी को अपना ज्योति–स्तंभ बनाओ। पढ़ा–पढ़ाया कुछ अंश तक पथ प्रदर्शक होता है पर सच्चे पथ–प्रदर्शक वे ही महापुरुष होते हैं, जो अपना नाम संसार में छोड़ जाते हैं। वे जीवन समुद्र में ज्योति–स्तंभ का काम देते हैं। ऐसे ही आत्मत्यागी–सत्यवादी और पक्षपात रहित महापुरुषों के चाहे वे जीवित हों या ऐतिहासिक, उनके पीछे चलो।'

अपनी बात इन्दौर से प्रारंभ करता हूं। विनोबाजी को लेकर इन्दौर सौभाग्यशाली रहा है। सन् 1960 में 23 जुलाई से 24 अगस्त तक विनोबाजी इन्दौर में रहे। विनोबाजी के हृदय में लोकमाता अहिल्याबाई के लिए विशेष आदर का भाव रहा है। इसी कारण उन्होंने इन्दौर को चुना। इस बारे में विनोबाजी कहते हैं, "जब कभी महापुरुषों के स्मरण का प्रसंग आता है, मैं अपने शरीर से उठकर उनके शरीर में दाखिल हो जाता हूं। अहिल्याबाई उपासनानिष्ठ राज्यकर्ता थीं। कवि मोरोपंत ने लिखा है, 'देवी अहिल्याबाई झालीस जगत्रयांत तू धन्य।' अहिल्यादेवी वास्तव में न्यायधर्म निरत थीं। हिंदुस्तान के इतिहास में वह एक बड़ा प्रयोग था कि राज्य कारोबार की धुरा एक उपासना परायण, धर्मनिष्ठ स्त्री के हाथ में आयी। अहिल्याबाई ने उस जमाने में वैद्यनाथधाम से जगन्नाथ पुरी का मार्ग बनवाया। यह उनकी व्यापक दृष्टि थी। विनोबा ने स्त्री–शक्ति के विषय में इन्दौर को डबल इंजन माना था। यहां अहिल्याबाई भी हैं और कस्तूरबा ट्रस्ट भी। विनोबा ने इन्दौर को सर्वोदय नगर बनाने की कल्पना रखी। उन्होंने इन्दौर में स्वच्छता अभियान चलाया और अश्लील पोस्टर के

# विनोबा
# विचार प्रवाह

## (अंतर्राष्ट्रीय संगीति)

(1 अगस्त 2020 से 11 सितम्बर 2020)


बाबा विनोबा भावे के 125 वें जन्मोत्सव पर समर्पित

# लघुकथा कलश

जनवरी-जून 2021

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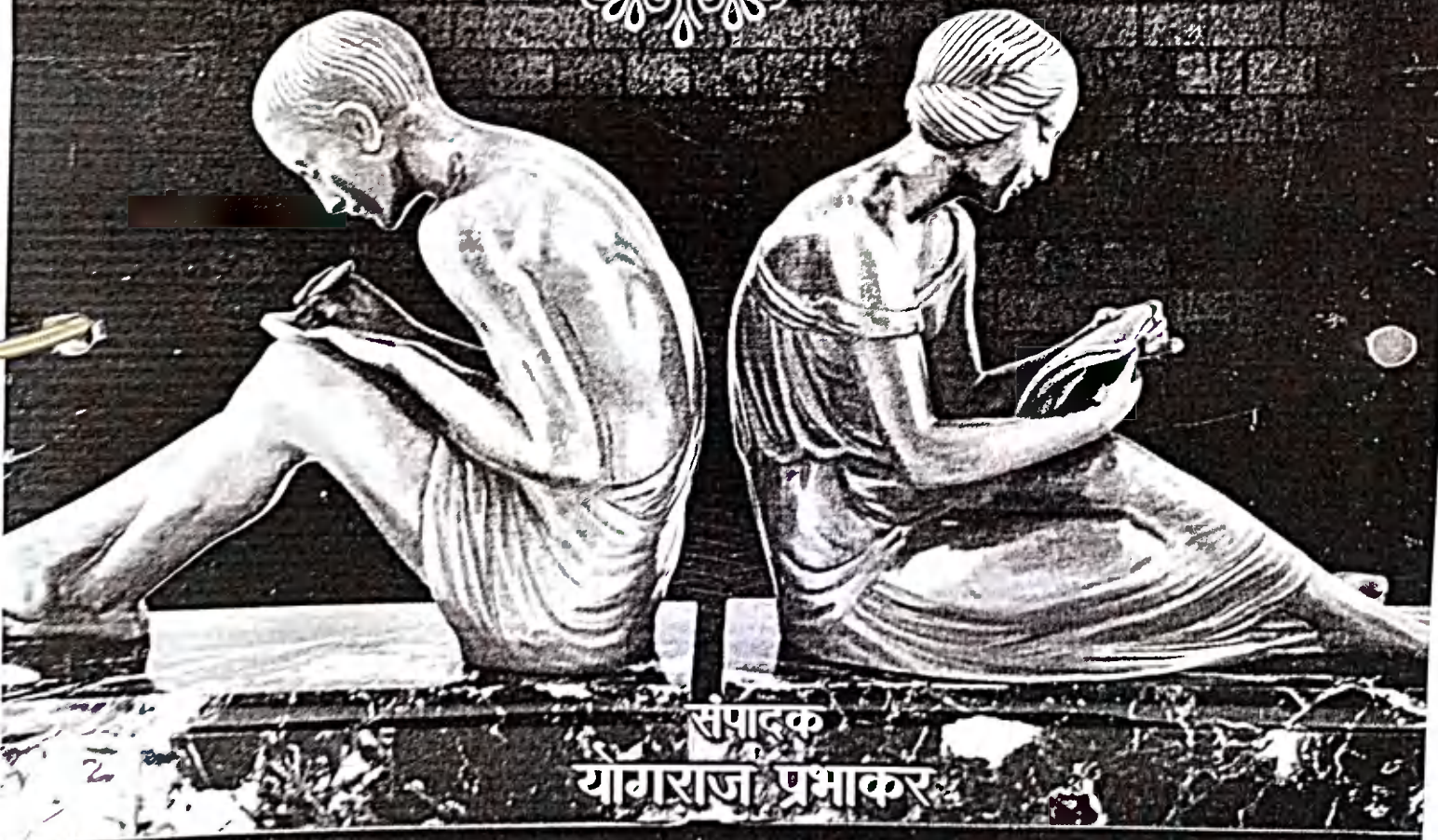
महाविशेषांक-२



संपादक
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# लघुकथा के सौम्य सत्याग्रही: डॉ॰ योगेंद्रनाथ शुक्ल

पुष्पेंद्र दुबे

विज्ञान युग में पूरा विश्व स्थूल से सूक्ष्म, कठोर से कोमल की ओर गति कर रहा है। स्थूल की प्रकृति कठोर होती है, वहीं सूक्ष्म का संबंध कोमलता से होता है। भौतिक जगत् में व्यवहार में दिखाई देने वाली कठोरता विज्ञान के कारण नेपथ्य में जा रही है और जीवन का संचालन करने वाले समस्त साधन कोमल हो चले हैं। कभी पहाड़ काटने, बाँध बनाने, सड़क निर्माण करने के लिए हाड़तोड़ परिश्रम करना होता था। अब वही कार्य कंप्यूटर और तकनीक के माध्यम से मनुष्य के हाथों की कोमल उँगलियाँ कर रही हैं। मनुष्य की उँगली के पोर के दबाव मात्र से पूरी सृष्टि नष्ट हो सकती है। आज विज्ञान के कारण इतनी ताक़त मनुष्य के पास आ गई है। मनुष्य ने पाँच सौ सालों में आकाश और पाताल में जितनी प्रगति की है उतनी पचास हज़ार साल में भी नहीं कर पाया। विज्ञान के प्रभाव से जीवन का कोई भी क्षेत्र अछूता नहीं रहा है। कभी महादेवी वर्मा ने छायावाद को 'स्थूल के प्रति सूक्ष्म का विद्रोह' कहा था। आज भौतिक जगत् में वही स्थिति निर्मित हुई है। प्रयोगवाद के लिए 'अज्ञेय' ने कहा था कि जिस प्रकार बासन अधिक घिसने से बर्तन अपना मुलम्मा छोड़ देते हैं, वैसे ही शब्दों ने अपना अर्थ खो दिया है। उनमें आधुनिक ज़माने के हिसाब से नया अर्थ भरने की आवश्यकता है। जिस प्रकार विज्ञान के क्षेत्र में छोटे-से छोटे सूत्र में प्रकृति को विघटित करने की शक्ति समाई हुई है, वैसे ही अध्यात्म में चित्तशक्ति के स्फोट का प्रयोग होता है। संस्कृत में सूत्र है 'क्रियोपरमे वीर्यवत्तरम्' अर्थात् कम-से-कम क्रिया में अधिक से अधिक परिणाम हासिल करना। विज्ञान और साहित्य दोनों पर ही यह सूत्र अक्षरश: चरितार्थ होता है। विज्ञान की सूक्ष्म क्रियाओं का परिणाम मनुष्य पर स्पष्ट परिलक्षित होता है। जिस प्रकार विज्ञान ने पूरी दुनिया को अपने में समेट लिया है, उसी प्रकार मनुष्य की भावनाओं ने सूक्ष्मातिसूक्ष्म रूप धारण कर व्यापक होने का प्रयास किया है। और इस व्यापक होने को साहित्य की अन्यतम विधा 'लघुकथा' में देखा जा सकता है। वैसे तो साहित्य सृजन किसी साधना से कम नहीं है। जब व्यक्ति अपने से अलग होता है, तब उसके द्वारा सृजित साहित्य जनसाधारण के हृदय का स्पर्श करता है। सुख-दु:ख के भीतर चलने वाले जीवन की समस्त भावनाओं को वाणी प्रदान करना 'लघुकथाकार' अपना दायित्त्व मानता है। डॉ॰ योगेंद्रनाथ शुक्ल ने इस दायित्त्व का निर्वाह बहुत निष्ठा से किया है। उनकी निष्ठा का पता इसी बात से लगता है कि उन्होंने लघुकथा को हमेशा ताज़गी और नई दिशा प्रदान की है। कबीर के 'एकै साधे सब सधे, सब साधे सब जाए' के सिद्धांत पर चलते हुए डॉ॰ शुक्ल ने लघुकथा विधा में ही अपनी लेखनी चलाई है। यद्यपि उन्होंने आलेख, व्यंग्य, कविताएँ भी लिखी हैं, परंतु उनका मन 'लघुकथा' में अधिक रमा है। उनकी लघुकथाओं का जन्म आपस की चर्चाओं से ही हो जाता है। इसलिए उनमें कहीं पर भी बनावटीपन नहीं लगता। कहने का आशय यह है कि शुक्ल जी बातचीत में से सार ग्रहण करने

*देश की सभी प्रमुख पत्र-पत्रिकाओं में डॉ॰ शुक्ल द्वारा रचित 550 से लघुकथाएँ, कहानियाँ, शोधपरक आलेख, कविताएँ, संस्मरण, व्यंग्य प्रकाशित हो चुके हैं। उनकी साहित्य साधना के लिए उन्हें डॉ॰ परमेश्वर गोयल लघुकथा शिखर सम्मान प्रदान किया गया है।*

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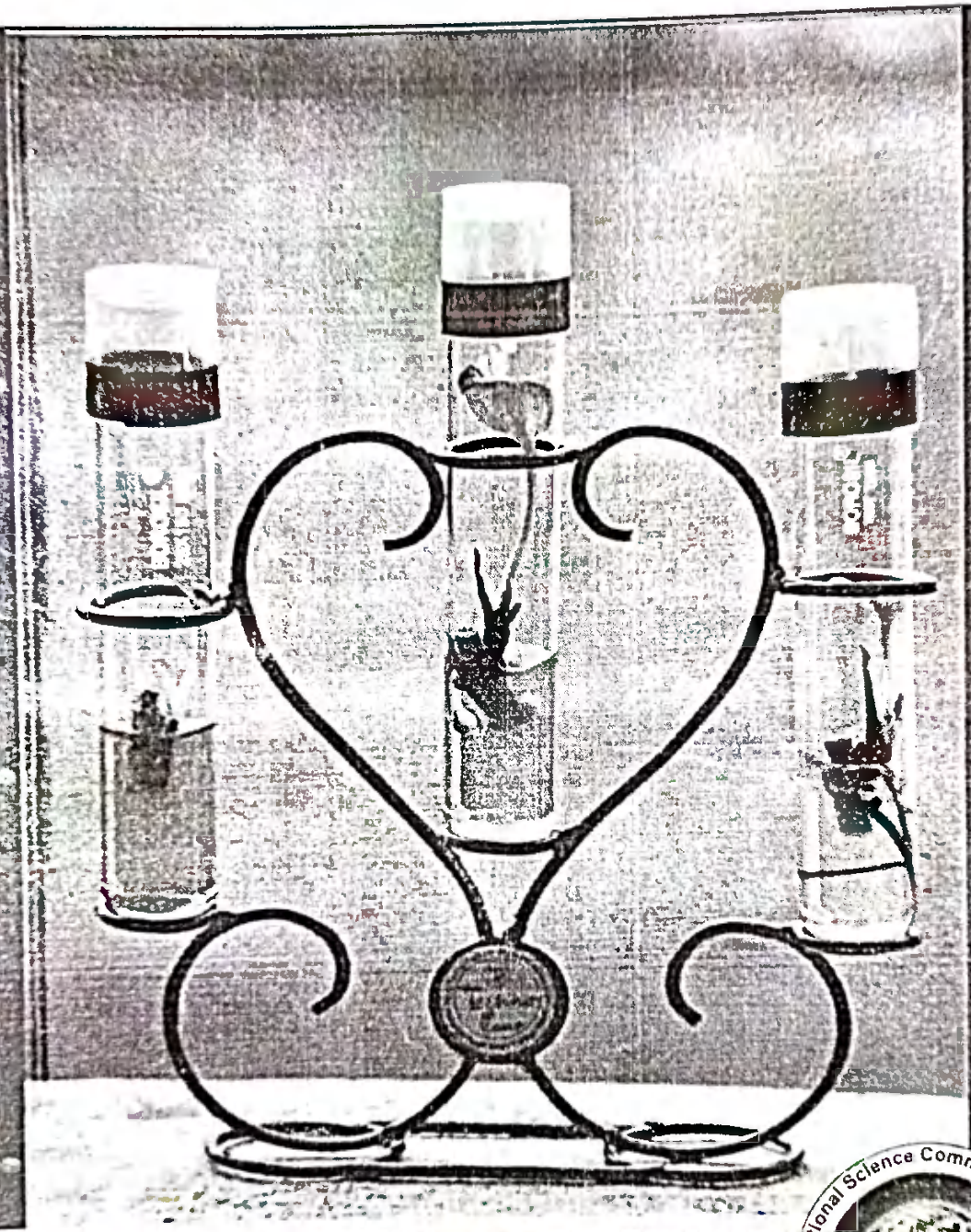

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# 8

# PECTINASES: PRODUCTION AND APPLICATIONS FOR FRUIT JUICE BEVERAGES

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## 8.1 Introduction

Pectin is a complex polysaccharide and presents prominently in the middle lamella of plant cell wall (Kertesz, 1951). Pectin forms about one-third of the cell wall dry substances of dicotyledonous plants (Jarvis et al., 1988). The pectic substances play an important role in cell adhesion and provide mechanical strength to the cell wall (Jarvis, 1984). Pectic substances are acidic heteropolysaccharides with molecular masses ranging from 23 000 to 360 000 D (Newbold and Joslyn, 1952). They occur in varying amounts in all higher plant tissues. Fruits and vegetables contain varying amount of pectin from 0.2% to 0.5% in tomatoes to 30% to 35% in citrus peel (Tapre and Jain, 2014). The presence of pectin is crucial to the formation of fruit products from clear juices to jams, jellies, and marmalades. The first information on water-soluble jellying substances in fruits was published by Vauquelin (1790). In 1825, Braconnot showed de-esterification of pectin to pectic acid and 162 years later, Vennigerholz and Wales (1987) showed that digestion of the tissues with pectolytic enzymes leads to dissolution of the middle lamella and thereby cell separation.

Pectic enzymes have been used as bulk heterogenous preparations by fruit processing industries. Mostly these industries use fungal enzymes supplied by Gist-Brocades, Novo-Nordisk, Biocon, A.T.P, ABM Sturge, Genencore, Amano, and Shin-Nihon (Pilnik and Voragen, 1993). However, relatively pure preparations of various immobilized pectic enzymes have also been employed in the form of bioreactor for industrial applications (Nighojkar et al., 1995).

This chapter deals with the occurrence, classification, structure, composition and degree of esterification of pectin, commercial pectin

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preparations, various pectic enzymes, their classification, production of pectin methylesterase, endopolygalacturonase, and their applications in fruit beverages.

## 8.2 Pectin

### 8.2.1 Definitions Related to Pectins

The family of oligosaccharide and polysaccharide having common features and rich in galacturonic acid (65% as stipulated by Food and Agriculture Organization) is termed as pectin (Brent et al., 2001). However, in the early years, there was a lot of confusion regarding the naming of pectic substances. The definitions of these complex substances as given by a committee of the American Society (1944) are generally accepted and the same definitions have been followed. These are as follows:

- Protopectin is the name given to the water-insoluble parent pectic substance which occurs in plants, and from which pectic substances are produced.
- Pectic substances are a group designation for those complex colloidal carbohydrates which occur in or are prepared from plants and contain a large proportion of anhydrogalacturonic acid units. The carboxyl group of polygalacturonic acid may be partly esterified by methyl groups and partly or totally neutralized by one or more bases.
- Pectinic acids are the term used to designate colloidal polygalacturonic acids containing more than a small proportion of methylester groups. Pectinic acids under suitable conditions are capable of forming gels with sugar and acid; if the methoxyl content is low then gel formation may take place with certain ions. The salts of pectinic acids are either normal or acid salts of pectic acids.
- Pectin or pectins designate those water-soluble pectinic acids of varying methylester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions.
- Pectic acid is the name applied to pectic substances composed of colloidal polygalacturonic acid and is essentially free of methyl ester groups. The salts of pectic acid are either normal or acid pectates.

A group of definitions given by Doesburg (1965) are considered by some workers to be more complete (McCready, 1970). However, other workers considered the definitions given by the American Society (1944) (Kertesz, 1951; Fogarty and Kelly, 1983) to be more appropriate.

## 8.2.2 Occurrence

Mangin (1888) was the first to suggest the presence of pectin in the middle lamella of plant cells. Development of pectic substances appears to occur at the time when all nuclei are beginning to divide (Allen, 1901). A pectin-containing membrane divides the cell into two parts. As the division continues, cellulosic membranes are formed with pectic material in the middle lamella. Earlier, the researches on pectic substances were based on staining with ruthenium red. Hydroxamic acid test in situ (McCready and Reeve, 1955) was also used for studying location of pectin in plants. However, the fine structure of cell wall was revealed with electron microscopy studies (Frey-Wyssling and Muhlethaler, 1965). Pectic substances are now known to be present without exception in and between the cell walls of photosynthetic green plants. The content of pectin varies from plant to plant and tissue to tissue from as low as 0.1%–0.5% in tomatoes to 32% in lemon rind (Whistler and Smart, 1953). The young actively growing plant tissues are particularly high in pectin (Northcote, 1963).

There are two theories regarding role of pectin in cementing plant cells together. The pectate theory, based on the in vitro behavior of pectic substances and experiments with fruit tissue, suggest that the plant cells are held together by calcium pectate gels. This theory is supported by the observation that the chelating substances liquefy calcium pectate gels and also promote disintegration of fruits and some vegetables. Treatment with calcium salts results in firming of tomatoes, apples, and other fruits. The theory is weakened by the fact that only a small amount, if any, of the pectic substances in mast fruits and vegetables contain calcium pectate or pectic substances having low degree of esterification. The other theory is the protopectin theory proposed by Joslyn (1962).

The important features of the theory are:

- bonding to polyvalent ions, for example, calcium, magnesium, and iron, thus leading to insolubility of low esterified pectic polysaccharides and reduction of swelling of the highly esterified pectic materials;
- bonding of carboxyl groups of pectin and the hydroxyl groups of other cell wall constituents, for example, cellulose, hemicelluloses;
- salt bonding between the carboxyl groups of the pectic substances and the basic groups of proteins;
- secondary valency bonding between pectic polysaccharides or with other cell wall constituents; and
- mechanical entwining of pectic polysaccharides with each other and with other polymers in the cell wall.

### 8.2.3 Classes of Pectin

Pectin molecule is a rhamnogalacturonan chain consisting of α-D-1,4-galacturonan region ("smooth region") interrupted by insertion of 1,2 linked α-L-rhamnosyl residue (Selevndran, 1985). Side chain composed of neutral sugars are attached by glycosidic linkages to C3 and C4 of the rhamnose units and C2 and C3 of galacturonic acid units giving rhamnogalacturonan portion of the pectin backbone a "hairy" character (De Vries et al., 1986). A large number of –COOH groups of the galacturonic acid monomers in the main chain ("smooth region") are methyl esterified.

Generally, three classes of pectin have been described depending on the procedures used to extract them from cell walls. These are water-soluble pectin extractable with water or dilute solutions; chelator-soluble pectins extractable with solutions containing calcium ion chelating agents such as EDTA, CDTA (1,2-cyclohexanediaminetetra-acetic acid), or hexametaphosphate, and protopectins that are brought into solution with alkali solution or hot dilute acids (Van Buren, 1991). Protopectins are secured in the primary cell wall matrix by the acid and/or alkali-labile bonds. Ryden and Selvendran (1990) have shown that although a large part of protopectin can be solubilized by 0.05 M sodium carbonate, still a small fraction remains insoluble even after extraction with 4 M potassium hydroxide. Selevndran (1985) has suggested that the first two classes of pectins viz. water soluble and chelator soluble are derived from the middle lamella. The protopectin chain may have a part of it embedded in the cell wall, with the rest extending into the middle lamella.

The proportions of these three types of pectin vary considerably in different tissues. Most of the pectin in carrots and snap-bean pods (Sajjaanantakul et al., 1989) is of the chelator-soluble type. In ripe and even senescent apples most of the pectin is of protopection type (Massey et al., 1964). In some other ripe fruits, such as freestone peaches (Postlmayr et al., 1956), most of the pectin is of the water-soluble type, while in ripe clingstone peaches, approximately equal proportion of all three types of pectin has been found. In tissues such as carrots, potatoes, and snap-bean pods with high proportions of chelator-soluble pectin, the infusion of chelators into the tissue results in dramatic losses of cohesion (Linehan and Hughes, 1969). Tissues such as beetroot, with a high proportion of protopectin, show little loss of cohesion when treated with chelating agents.

The water-soluble and chelator-soluble pectins are typically composed of mainly galacturonic acid residues with about 2% rhamnose and 10%–20% neutral sugar. The distribution as well as the number of free carboxyl groups may be important in affecting whether pectin is water soluble or chelator soluble. The protopectins, particularly if

they are extracted with alkali, have neutral sugar content (Selevndran, 1985), mainly galactose and arabinose. Commercially prepared pectins often resemble water-soluble and chelator-soluble pectins in their composition, but it is likely that many of their neutral sugars have been removed by hydrolysis during extraction.

It seems that the major contribution to intercellular adhesion comes from the chelator-soluble fraction and the protopectin. In general, softening during ripening (Massey et al., 1964) or heating (Van Buren et al., 1960) is accompanied by a loss of protopectin and an increase in water-soluble pectin.

Pectins can also be classified depending on the degree of esterification into two classes: high-methoxyl pectin with degree of esterification >40%–50% (Hercules, Inc. 1985) and low-methoxyl pectins, product of further regulated acid, alkali, or enzyme treatment of high-methoxyl pectin with degree of esterification <40%. Both the classes of pectin form gels under different set of conditions and have different properties.

## 8.2.4 Pectin Structure and Composition

Recent studies on pectin structure indicate that although pectin has been studied from different fruits since last over two decades, still their structure and composition varies a lot. Chan et al. (2017) have shown that various fruit sources used for commercial pectin production such as apple pomace, citrus peel, sugar beet pulp, banana peel, mango peel, watermelon rind, tomato peel, sunflower head, papaya peel, passion fruit rind, plum pomace, rapeseed cake, and sisal waste have content of pectin ranging from 1.8% in rapeseed cake to 83.5% in tomato peel.

In the 1950s, pectic substances were regarded as a triad of polysaccharides consisting of mainly galacturonan along with some araban and galactan (Whistler and Smart, 1953). D-Galacturonic acid units joined by means of α—(l→4) glycosidic linkages form the main uronide chain of pectic polysaccharides. Rhamnose introduces a kink in the otherwise straight chain by joining to the reducing end of the uronide by (l→2) linkages and to the nonreducing end of the next uronide unit by (l→4) bonds. However, the mole percent of rhamnose varies depending on the type of pectin (Ryden and Selvendran, 1990). Often, arabinan, galactan, or arabinogalactan side chains are linked (l→4) to the rhamnose. In addition, other sugars, such as D-glucuronic acid, L-fucose, D-glucose, D-mannose, and D-xylose are sometimes found in the side chains. Atmodjo et al. (2013) in his review paper have explained the presence of three major pectic polysaccharide, homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II

(RG-II). Approximately 65% of the pectin is HG, a homopolymer of (α-1,4) linked D-galacturonic acids. Partial methyl esterification at sixth position and lesser acetylation at $O_2$ and $O_3$ position gives HG.

The RG-II has a HG backbone substituted with side chain of an octasaccharide and a monosaccharide linked to an $O_2$ or disaccharide linked to $O_3$ on the HG backbone. RG-II constitutes 10% of the pectin and is more complex and structurally conserved as compared to RG-I which constitute 20%–25% of pectin. RG-I has a repeat backbone of highly acetylated ($O_2$ or $O_3$) galacturonic acid residues. The structural data indicate that HG, RG-I, and RG-II are connected by covalent linkages via their backbone forming an interconnected pectin structure in the cell wall. However, as yet the complete structure of native pectin from cell wall has not been isolated.

Pectic substances differ in chemical properties from other polysaccharides primarily because of the presence of large amounts of carboxyl groups, which are partially esterified with methanol. The degree of esterification/methoxyl content may vary with the pectin source and method of extraction used. When all carboxyl groups in polygalacturonic acid are esterified, the methoxyl content is 16.32, that is, degree of esterification is 100%. However, some of the carboxyl groups are free acids and some are neutralized with various ions. Some of the hydroxyl groups on $C_2$ or $C_3$ may be acetylated at a rate of 0.18%–2.5% (McComb and McCready, 1957). Pippen et al. (1950) showed the importance of acetyl group in affecting gelling properties; one acetyl group per eight galacturonic acid units prevents pectin from jelly formation.

The molecular weight of pectic polysaccharides from various sources has been determined. The molecular weight in citrus, 23 000–71 000 D (Porwal and Chakravarti, 1970); apple, plum, and pear, 25 000–35 000 D; and apple and lemon, 200 000–360 000 D (Newbold and Joslyn, 1952) has been reported. The values are affected by the mode of extraction and the technique of estimating the molecular weight. Pectin solutions have high positive optical rotation which is dependent upon degree of polymerization. D-Galacturonic acid has a specific rotation $(\alpha)^{20}$ of $+51.7^{O}$ whereas pectic polysaccharides have an average $(\alpha)^{20}$ of about $+235^{O}$.

Our present knowledge of modification of pectin structure during growth, differentiation, and in response to environmental stimuli is still expanding. However, it is pertinent to mention that pectic polysaccharide plays a major role as regards to cell strength, cell adhesion, stomatal function, and defense response (Caffall and Mohnen, 2009). The pectic polysaccharide also finds applications in industries and medicine which varies according to the modification in their structure.

### 8.2.5 Degree of Esterification of Pectin

An important factor in characterizing pectin chains is the degree of esterification of the uronide carboxyl groups with methanol. Pectins might be formed initially in a highly esterified form, undergoing some de-esterification after insertion into the cell wall or the middle lamella (Van Buren, 1991). Depending on species, tissue, and maturity, the degree of esterification of pectins may vary from 60% to 90%. The water-soluble pectin and protopectins have a higher degree of esterification than chelator-soluble pectin. Degree of esterification is also a measure of the viscosity of the solution. Solution of fully de-esterified pectin does not change its viscosity appreciably with change in pH, but the viscosity of low-ester pectin solution is markedly pH dependent (Doesburg, 1965).

An important character of pectin is its ability to form gels. The gel-forming ability and the gel characteristics are also dependent on the degree of esterification of the pectin used. Both high-methoxyl and low-methoxyl pectins form gels. In general, a pectin with 70% degree of esterification or more (methoxyl content 11.4%–13%) forms a rapid set gel with acid and sugar, whereas, a pectin with degree of esterification between 50% and 70% (methoxyl content 8.75%–10.5%) forms a slow set gel with acid and sugar. Pectins with degree of esterification less than 50% precipitate in acid media and are not used to make high solid gels. However, they are advantageous for preparation of low sugar or sugar-free, low calorie gels with addition of calcium to pectinic acids under controlled conditions. Pectinic acids are largely used in foods and beverages, particularly in diets for diabetics, where sugar is replaced by nonnutritive sweeteners.

### 8.2.6 Commercial Pectins

Commercial pectins are used in the manufacture of jams, jellies, and marmalades. Pectin being a natural product, one envisages some variation in grade and jellying capacity between one batch and another. Normally, different lots of pectin are blended and dextrose is added as a diluting agent in order to obtain a product of fixed grade. To control setting time, sodium citrate is sometimes added as stabilizer. The pectin gels, having high sugar content are generally prepared from high-methoxyl pectins and those holding a low sugar content are produced from low-methoxyl pectins and calcium salts. High-methoxyl pectin is used chiefly in the preparation of jams. They are fast setting, high temperature jellying agents, whereas partial de-esterification gives slow setting pectins. The slow rate of gelation allows enough time for air bubbles trapped in the cooling solution during pouring

to escape from the container, and for other operations; for example, filling, capping, and labeling to be completed before setting, so that the process is not disturbed (Pilgrim et al., 1991). Sugar, for example, sucrose or a similar carbohydrate, in adequate amount acts as a dehydrating agent for the pectin molecules, thereby permitting closer association between the polymeric chains. Normally over 50% sugar is adequate for gel formation. The high molecular weight also favors gel-forming properties.

## 8.3 Pectic Enzymes and Their Classification

Pectic polysaccharides occur universally in the plant world. The enzyme system capable of degrading these structures is not only varied in their mode of action, but is also widespread in their distribution. Pectic enzymes are produced by plants, many bacteria, and fungi as well. However, from animal cells, only polygalacturonase has been identified; as in snails (Ehrlich, 1932).

Endogenous pectic enzymes therefore can produce important textural changes in fruits and vegetables during ripening and storage. The necessity to activate or inactivate them often has a decisive influence on processing steps in the manufacture of the products derived from fruits and vegetables. Microbial pectic enzymes serve functions in plant pathology and fermented foods but are also produced industrially as processing aids in the food industry. Pectic enzymes purified to well-defined activities are used in pectin analysis, structural research of pectins, and cell wall studies.

Pectinases or pectinolytic enzymes have been classified on the basis of their reaction to different pectic substances (Sharma et al., 2013) or on the basis of their reaction mechanism (Jayani et al. 2005). The pectic enzyme that hydrolyze pectin has been categorized in two groups.

### 8.3.1 Esterase

Pectin methylesterase (EC.3.1.1.11) also called pectinesterase (systematic name: pectin pectyl hydrolase) cleaves methanol from esterified carboxyl groups to yield low-methoxyl pectin and polygalacturonic acid. High-methoxyl pectin is the preferred substrate.

Pectin acetylesterase (EC. 3.1.1.6) hydrolyzes acetyl esters in HG regions of pectin (systematic name: acetic-ester acetylhydrolase). Other names in common use include C-esterase, acetic ester hydrolase, chloroesterase, *p*-nitrophenyl acetate esterase, and Citrus acetylesterase.

## 8.3.2 Depolymerases

The depolymerizing enzymes are either hydrolyzing pectin or pectic acid (polygalacturonic acid) or perform transelimination reaction on unsaturated polymethyl digalacturonates or pectic acid (Jayani et al., 2005). Depolymerases can also be divided into three subgroups.

1. acting on pectin;
2. acting on pectic acid (polygalacturonic acid); and
3. acting on oligo-D-galactosiduronates.

Each subgroup can be further divided into two subsubgroups; hydrolases and lyases, which can be further subdivided into endo- and exo-enzymes depending on the mode of action.

### *8.3.2.1 Acting on Pectin*

#### Polymethylgalacturonase

Endopolymethylgalacturonase (PMG) performs random hydrolysis of highly esterified pectin to form oligogalacturonates.

Exo-PMG hydrolyses pectin in a sequential fashion from the terminal end liberating monogalacturonates.

#### Polymethylgalacturonate Lyase

Endopolymethylgalacturonate lyase (PMGL) (EC. 4.2.2.10) causes random cleavage in pectin by a transelimination process, forming a double bond between C4 and C5 of the galacturonic acid residues at the nonreducing end (systematic name: poly (methyl galactosiduronate) endo-lyase) also called as endo-pectin lyase. It carries out random transelimination of unsaturated poly (methyl) D-digalacturonate releasing unsaturated methyl oligo galacturonates.

Exo-poly methyl D-galactosiduronate lyase: performs terminal transelimination of unsaturated poly-(methyl-D-digalacturonate) releasing unsaturated methyl monogalacturonates (systematic name: poly(methoxygalactosiduronate) exo-lyase) also called exo-pectin lyase.

### *8.3.2.2 Acting on Polygalacturonic Acid or Pectic Acid*

#### Polygalacturonase

Endopolygalacturonase (PG), EC. 3.2.1.15), hydrolyzes polygalacturonic acid in a random fashion [systematic name: poly (1, 4 α D-galactosiduronate) glycanohydrolase] releasing oligogalacturonides.

Exo-PG-1, (EC. 3.2.1. 67), hydrolyzes polygalacturonic acid releasing D-galacturonate, that is, hydrolyses successive bonds (systematic name: poly(1,4-α-D-galactosiduronate) galacturonohydrolase).

Exo-PG-2, (EC. 3.2.1.82), hydrolyses polygalacturonic acid from nonreducing-end releasing digalacturonate, that is, hydrolyses alternate bonds [systematic name: poly (1,4-α-D-galactosiduronate) digalacturonohydrolase].

#### Polygalacturonate Lyase

Endopolygalacturonate lyase (PGL), (EC. 4.2.2.2), causes random cleavage in polygalacturonic acid by a transelimination process [systematic name: poly-(1,4-α-D-galactosiduronate) endolyase] commonly called as endopectate lyase.

Exopolygalacturonate lyase, (EC. 4.2.2.9), causes sequential cleavage in polygalacturonic acid by transelimination process [systematic name: poly (1,4-α galactosiduronate) exo-lyase] commonly called as exopectate lyase.

### *8.3.2.3 Acting on Oligo-D-Galactosiduronates*

#### Oligogalacturonase

Oligogalacturonase (OG) hydrolyzes oligo-D-galactosiduronate [systematic name: oligo-D-galactosiduronate hydrolase].

#### Oligogalacturonate Lyase

Oligogalacturonate lyase (OGL) (EC. 4.2.2.6) causes cleavage of unsaturated oligo-D-galactosiduronate by a transelimination process [systematic name: oligo-D-galactosiduronate lyase].

## 8.4 Production System for Pectinases

### 8.4.1 Production From Plant Sources

Pectic enzymes have been widely studied in plants mainly fruits as they are expressed during fruit ripening. Since pectin occupies the middle lamella of plant cell wall, high salt concentration is required for their isolation. For this purpose, 10% NaCl is being used (Shrivastava et al., 1994). The extraction and complete purification needs to be done in cold conditions as plant enzymes are thermolabile. Looking to increased advantages of microbial enzymes as compared to plant enzymes, mainly the cost effectiveness, thermostability, shorter purification time, reproducibility of results, and industrial applications, nowadays the pectic enzymes are being isolated from microbial sources.

### 8.4.2 Production From Microbial Sources

Solid-state fermentation (SSF) and submerged fermentation (SmF) processes are used extensively for pectinolytic enzyme production

using various microorganisms. Bacteria, yeasts, and fungi produce pectic enzymes both under SmF and SSF conditions. Bacterial enzymes are mainly alkaline and thermostable, while fungi majorly produce acidic pectinases (Favela-Torres et al., 2006). SSF is considered more suitable for fungi than for bacteria (Pandey 2003; Favela-Torres et al., 2006; Kumar et al., 2012). One of the pioneer works on pectinase production by SSF indicated that pectinase production by *Aspergillus niger* was about 11 times higher in SSF than in SmF (Solis-Pereira et al., 1993). Moreover, endopolygalacturonase production by *Paecilomyces clavisporus* was 28 times higher per gram of solid substrate in SSF as compared to per ml of culture medium in SmF (Souza et al., 2003). SSF is a process that occurs in the absence or near absence of water or any liquid. It is attributed that the increased polygalacturonase production in SSF, as compared to that in SmF is due to the expression of a second polygalacturonase (PG II), which is biochemically different from the one produced in SmF (Niture and Pant, 2004).

Agro-industrial waste is used as solid substrate for fermentative metabolite production using fungi. The fungi are capable of growing over and utilizing agro waste as it is their natural habitat. This makes them more interesting for their use in SSF processes. Fungi are more adapted to SSF because their hypha can grow on the agro residue surface, penetrating into the inter particle spaces, and colonizing the solid substrates. In contrast, in SmF, the nutrients and microorganisms are both submerged in water thus making it suitable for bacterial isolates (Graminha et al., 2008). However, SSF processes can be well manipulated and managed for scarcely produced pectinolytic enzymes using bacterial culture (Gupta et al., 2008; Kashyap et al., 2003).

Maldonado and Stresser de Saad (1998) reported 4 times higher pectin methylesterase production by *A. niger* in SSF than in SmF system. Also, polygalacturonase production was reported 6 times higher in SSF than in SmF system over a shorter time period for enzyme production. Patil and Dayanand (2006) compared SSF and SmF processes for *A. niger* pectinase production and reported higher production by SSF. SSF has several advantages over SmF, which are summarized in Table 8.1.

## 8.5 Pectinolytic Microorganisms

More than 30 different genera of bacteria and fungi have been used for the production of polygalacturonase (Favela-Torres et al., 2005). *Erwinia, Bacillus, Saccharomyces, Kluyveromyces, Aspergillus, Penicillium, Fusarium,* and *Rhizopus* have been the genera most frequently studied in the last 15 years, of which strains of *Aspergillus, Penicillium,* and *Erwinia* are mainly used for enzyme production studies.

## Table 8.1 Advantages and Disadvantages of SSF Over SmF (Raimbault, 1998; Holker et al. 2004; Couto and Sanroma, 2006)

| Advantages | Disadvantages |
|---|---|
| • Higher productivity<br>• Better oxygen circulation<br>• Low-cost media<br>• Less chance of contamination<br>• Absence of foam formation so no requirement of antifoaming agent<br>• It resembles the natural habitat for several microorganisms<br>• Less effort in downstream processing<br>• Simple technology<br>• Less energy demand for heating<br>• Fermentation of water insoluble material<br>• Requires smaller reactor volume<br>• Catabolite repression is negligible | • Mixing of nutrients not uniform<br>• Difficulties on scale-up<br>• Problems with heat build-up<br>• Higher impurity product<br>• Recovery cost is high<br>• Difficult control of process parameters |

Pectinolytic enzymes are widely distributed among bacteria, fungi, higher plants, parasitic plants, and some plant parasitic nematodes (Shrivastava et al., 1994; Jayani et al., 2005). Several fungal strains have been used in SSF for industrial production of pectinolytic enzymes. The widely used *Aspergillus* species for polygalacturonase production are *Aspergillus sojae* (Heerd et al., 2012; Demir and Tari, 2014), *A. niger* (Dhillon et al., 2007; Ruiz et al., 2012; Darah et al., 2013; Trentini et al., 2015), *Aspergillus fumigatus* (Sandri et al., 2015), *Aspergillus sydowii* (Singh and Mandal, 2012), *Aspergillus tubingensis* (Tai et al., 2014), *Aspergillus carbonarius* (Nakkeeran et al., 2011), *Aspergillus niveus* (Maller et al. 2011), *Aspergillus awamori* (Botella et al., 2005; Diaz et al., 2012), and *Aspergillus oryzae* (Meneghel et al., 2014). Some sources of pectin degrading microorganisms have been given in Table 8.2.

Fungi, such as *A. tubingensis* (Patidar et al., 2016a), *A. niger* (Jiang et al., 2013; Joshi et al., 2006; Van Alebeek et al., 2003; Hasunuma et al., 2003), *Aspergillus aculeatus* (Duvetter et al., 2005), *Fusarium asiaticum* (Glinka and Liao, 2011), *Penicillium notatum* (Gayen and Ghosh, 2011)

## Table 8.2 Sources of Pectinolytic Microorganisms

| Source | Microorganism | Reference |
|---|---|---|
| • Soil of plum orchid waste site | *Aspergillus* sp. | Sunnotel and Nigam (2002) |
| • Decaying orange peel | *Asergillus fumigatus* | Phutela et al. (2005) |
| • Agro waste dumping pit soil | *Aspergillus niger* | Patil and Dayanand (2006) |
| • Citrus fruit peels | *Aspergillus niger* | Martos et al. (2009) |
| • Agricultural waste | *Aspergillus* sp. *Penicillium* sp. | Zeni et al. (2011) |
| • Rotten orange | *Aspergillus niger* | Darah et al. (2013) |
| • Rotten mango | *Aspergillus niveus* | Maller et al. (2011) |
| • Oak (Quercus spp.) | *Penicillium pinophilum* | Ruiz et al. (2012) |
| • Olive paste and olives | *Aspergillus niger* | Sanchez et al. (2015) |
| | *Aspergillus fumigatus* | |
| • Soil of fruit processing site | *Aspergillus niger* | Patidar et al. (2017) |
| | *Aspergillus tubingensis* | Patidar et al. (2016b) |
| • Pulp and paper mill, paper mulberry bark, vegetables and fruits | *Erwinia carotovora* | Sittidilokratna et al. (2007) |
| | *Erwinia chrysanthemi* | |
| | *Bacillus* sp. | |
| • Agricultural and vegetable waste | *Bacillus* sp. | Soares et al. (1999) |
| • Rotten vegetable | *Bacillus licheniformis* | Rehman et al. (2014) |
| • Soil sample | *Bacillus pumilus* | Sharma and Satyanarayana (2006) |
| • Soil, water, rotten fruit and vegetables | *Bacillus* sp. | Tariq and Latif (2012) |
| • Soil contaminated with effluents of paper and pulp industry | *Bacillus subtilis* | Kaur et al. (2011) |
| • Decomposing kitchen waste | *Bacillus subtilis RCK* | Gupta et al. (2008) |
| • Glass fibrer microfilters | *Geotrichum klebahnii* | Zapata and Voget (2012) |

*Botrytis cinerea* (Valette-Collet et al., 2003) and *Fusarium oxysporum* (Miller and Macmillan, 1971), and bacteria *Xanthomonas* sp. and very few yeast like *Saccharomyces cerevisiae, Candida boidinii* (Nakagawa et al., 2000; Jayani et al., 2005; Kohli et al., 2015) have been reported to produce pectin methylesterase enzymes.

However, few reports are available to show the use of microorganisms in SSF for pectin methylesterase production. *Aspergillus* sp. and *Penicillium* sp. have been used in SSF for pectin methylesterase production (Maldonado and Strasser de Saad, 1998; Taragano and Pilosof, 1999; Joshi et al., 2006; Gayen and Ghosh, 2011). Pectin methylesterase and polygalacturonase production has been recently reported in SSF by *A. tubingensis* MP30 and *A. niger* AN07, respectively (Patidar et al., 2016b, 2017).

## 8.6 SSF Process Conditions

SSF depends on a number of parameters, which are as follows:

### 8.6.1 Solid Medium

The yield of pectinolytic enzyme can be affected by the choice of medium. Also, it affects the production cost of the enzyme. In this regard, the agro-industrial waste is found to be economical and environmental friendly choice as a solid medium for high enzyme production.

The agricultural and food processing waste consists of a large amount of organic matter with high nutrient value. Thus, agricultural waste products and also wastes from biorefineries have been explored for abundant possibilities as novel low-cost enzyme production media. They are a good source of solid substrate and are used in SSF for production of polygalacturonase and pectin methylesterase enzymes. Agro wastes utilized as solid medium in SSF are listed in Table 8.3.

*Wheat bran*: According to a report of United States Department of Agriculture (USDA, 2013), 655,270,000 tons of wheat was produced in the period 2012–13 in the world. About 15%–20% of wheat bran is reported to be discarded during wheat flour production process (Dobrev et al., 2007, Demir and Tari, 2014), thus making it a sustainable by-product and one of the most popular agro-industrial residue for the microbial production of industrially important enzymes in SSF (Balkan and Ertan, 2010; Freitas et al., 2006; Demir and Tari, 2014). Demir and Tari (2014) screened various agro industrial by-products and found wheat bran most suitable for the production of polygalacturonase in SSF by mutant strain of *A. sojae* without the addition of any nutritive or inducing supplement. Demir and Tari (2016) have reported the effect of moisture content on wheat bran thickness, which affects polygalacturonase production in SSF. Several researchers Hendges et al. (2011) and Heerd et al. (2012) used *A. niger* and Kashyap et al. (2003) and Gupta et al. (2008) used *Bacillus* sp. in SSF for polygalacturonase production using wheat bran as solid substrate. Wheat bran has been mixed with other agro-industrial waste such as an orange bagasse (1:1w/w), orange peel (7:3w/w), and soy bran (1:1w/w) for increased production of pectinolytic enzyme in SSF (Silva et al., 2005; Heerd et al., 2012; Castilho et al., 2000). Heerd et al. (2014) reported enhanced pectinase production by *A. sojae* in SSF using wheat bran supplemented with 30% sugar beet pulp as inducer.

*Rice husk*: Rice husk, the main by-product of rice processing industries, is also a widely used agricultural waste for production of various metabolites, thus reducing the environmental impact associated with improper disposal of it. Rice husk has been used in SSF for production of polygalacturonase and feruloyl esterase (Tai et al., 2014). Rice husk works as solid carrier and is mixed with 50 ml of pectin media consisting of (g/L) pectin, 10; sucrose, 20; $K_2HPO_4$, 1.0;

## Table 8.3 Agro-Industrial Wastes Utilized in SSF for Pectinolytic Enzyme Production

| Solid Substrate | Organism | Reference |
|---|---|---|
| Wheat bran | *A. sojae* | Demir and Tari (2014) |
| Wheat bran | *A. sojae* | Demir and Tari (2016) |
| Wheat bran | *A. niger* | Hendges et al. (2011) |
| Wheat bran and soy bran | *Aspergillus niger* | Castilho et al. (2000) |
| Wheat bran and orange peel | *Aspergillus sps.* | Heerd et al. (2012) |
| Wheat bran and sugar beet pulp | *Aspergillus sojae* | Heerd et al. (2014) |
| Wheat bran, rice bran, and apple pomace | *Bacillus* sp. DT7 | Kashyap et al. (2003) |
| Orange bagasse, sugar cane bagasse, and wheat bran | *Thermoascus aurantiacus* | Martins et al. (2002) |
| Orange bagasse and wheat bran | *Penicillium viridicatum* | Silva et al. (2005) |
| Apple pomace | *Aspergillus niger* | Berovic'and Ostrovers'nik (1997) |
| Strawberry pomace | *Lentinus edodes* | Zheng and Shetty (2000) |
| Grape pomace | *Aspergillus awamori* | Botella et al. (2005) |
| Pomelo citrus grandis | *Aspergillus niger* | Darah et al. (2013) |
| Citrus peel | *Aspergillus niger* | Dhillon et al. (2007) |
| Grape pomace and orange peel | *Aspergillus awamori* | Diaz et al. (2012) |
| Grape pomace and orange peel | *Aspergillus awamori* | Diaz et al. (2013) |
| Lemon peel | *Aspergillus niveus* | Maller et al. (2011) |
| Sunflower head | *Aspergillus niger* | Patil and Dayanand, (2006) |
| Lemon peel pomace | *Aspergillus niger* | Ruiz et al. (2012) |
| Rice husk | *Aspergillus tubingensis* | Tai et al. (2014) |
| Papaya peel | *Aspergillus tubingensis* | Patidar et al. (2016a, b) |
| Sugarcane bagasse | *Aspergillus niger* | Acuna-Arguelles et al. (1994) |
| Sugar beet pulp | *Bacillus gibsonii* | Li et al. (2005) |
| Mango peel | *Aspergillus foetidus* | Kumar et al. (2012) |
| Cassava bagasse | *Bacillus subtilis* | Swain et al. (2009) |
| Wheat bran | *Bacillus subtilis* | Gupta et al. (2008) |
| Bingtang sweet oranges | *Eupenicillium javanicum* | Tao et al. (2011) |

$NaNO_3$, 30; $MgSO_4 \cdot 7H_2O$, 5.0; KCl, 10; $FeSO_4 \cdot 7H_2O$, 0.01; pH 4.0 and subjected to SSF for polygalacturonase production (Tai et al., 2014).

*Sugarcane bagasse*: Sugarcane bagasse has been used as solid substrate for production of pectinolytic enzymes (Pandey et al., 2000). Pectinase production has been reported using bagasse impregnated with a medium containing pectin and sucrose (Acuna-Arguelles et al., 1994). Solis-Pereyra et al. (1996) impregnated sugarcane bagasse with high glucose concentration solution for pectinase production using

SSF in a packed-bed column fermenter. Maldonado and Strasser de Saad (1998) reported fermentation medium containing (g/L): urea, 0.3; $K_2HPO_4$, 0.65; $(NH_4)_2SO_4$, 1.26; $MgSO_4$, 0.02; $FeSO_4$, 0.029; pectin, 1.5; sugar cane bagasse as support, 23.1; pH 4.5 for solid-state production of polygalacturonase and pectin methylesterase.

*Sunflower head*: The semiarid tropical region of Northern India is having about 30,000 hectare area of sunflower crop mainly produced for its oil. The deseeded dried sun flower head is normally burnt to ash. Patil and Dayanand (2006) used deseeded sunflower head for pectinase production in SSF and SmF. Higher production of polygalacturonase was observed when the medium was supplemented with 6% sucrose as carbon source and 0.3% ammonium sulfate as nitrogen source in SSF.

*Papaya peel*: Papaya (*Carica papaya* L.) is grown in Australia, Bangladesh, Hawaii, India, Malaysia, Philippines, Sri Lanka, South Africa and a number of other countries in tropical America (OECD, 2005). India alone produces a total of 5,382,000 metric tons of papaya every year (IHD, 2013). Papaya is extensively used worldwide for fruit juice preparation, salad preparation and in cosmetics and medications due to its high vitamin content (vitamin A and C) and high fiber content (Ittimongkol et al., 2002; Almora et al., 2004; Silva et al., 2007). Papaya peel is approximately 20%–25% of its fruit weight and can be used as animal feed, but is generally discarded as by-product causing organic pollution in the environment (Koubala et al., 2014).

Papaya peel contains 45%–51% esterified pectin (Boonrod et al., 2006), which is a preferred substrate for production of pectin methylesterase using *A. niger* (Van Alebeek et al., 2003). Papaya peel has huge amounts of monosaccharides, pectin, and protein, which facilitates the growth of fungi (Chaiwut et al., 2010; Maran and Prakash, 2015). Hence, the use of papaya peel in SSF for production of pectin methylesterase and polygalacturonase helps to solve the pollution problem of papaya processing units. Papaya peel has been used along with 10% (w/v) orange peel for production of pectin methylesterase (Patidar et al., 2016b). It has also been used for polygalacturonase production in SSF using dried papaya peel and orange peel in ratio of 2:1 (Patidar et al., 2017).

Scanning electron microscopic (SEM) analysis of the fermented papaya peel used as substrate for the growth of *A. niger* for production of pectinase enzymes in the Authors lab is shown in Fig. 8.1. The fungal hypha and the fruiting bodies are evident and show luxuriant growth of the fungus on the untreated substrate.

*Mango peel*: Mango is one of the important tropical fruits cultivated in many tropical regions and distributed worldwide. In 2013, Indian production of mango fruits reached 18,002,000 metric tons on 2,500,000 ha area which is 35.8% of total fruit area (IHD, 2013). A major

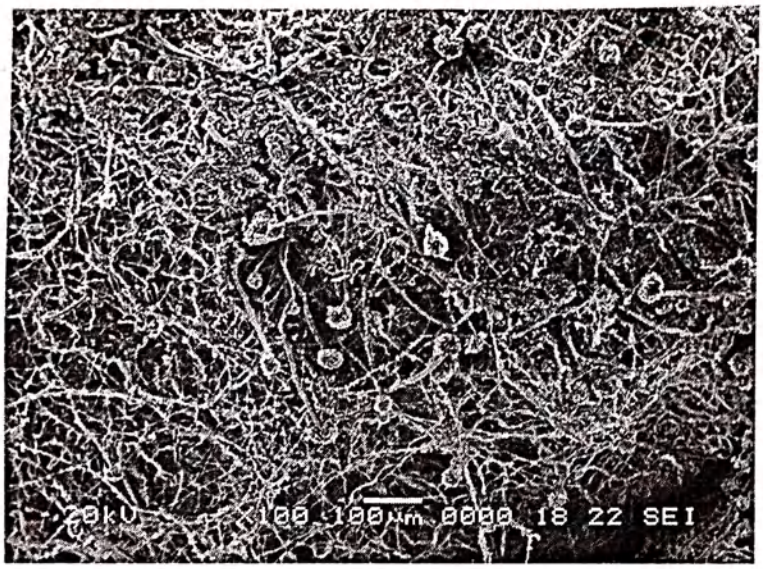


**Fig. 8.1** Scanning Electron Microscopic (SEM) analysis of the substrate. SEM type JEOL JSM 5600 showing dried samples of fermented papaya peel fixed on glass plate with 5-nm gold coating using Quorum—Q150TS.

by-product of the mango pulp industry is mango peel, constituting about 20%–25% of the mango fruit processing waste. Pectinase production has been achieved by *Aspergillus foetidus* using mango peel in SSF supplemented with salt solution consisting of (g/L) $(NH_4)_2SO_4$, 25; $MgSO_4$, 0.6; $FeSO_4$, 0.4; urea, 3; peptone, 5 and $KH_2PO_4$, 6 at pH 7.0 (Kumar et al., 2012).

*Citrus fruits*: The family of citrus fruits (Orange, Kinnow, Khatta, Lemon, Grapefruit, Malta, Mosambi, Sweet orange, etc.) is known to contain appreciable amounts of pectin (Alexander and Sulebele, 1980; Dhillon et al., 2007). Spain, the largest producer of citrus fruit in Europe with an output of 6 million tons/year produces approximately 800,000–900,000 tons of peel and pulp waste every year (IHD, 2013). The disposal is a big problem for the environment (IHD, 2013). Pectinolytic enzymes have been produced using lemon peel, orange peel, pomelo peel, etc. in SSF. Orange peel has been reported as an inducer for pectinolytic enzyme production in SSF and SmF due to high % of pectin (14.7%) (Nighojkar et al., 2006; Zhou et al., 2011). Orange bagasse and wheat bran (1 : 1w/w) by Silva et al. (2005) was utilized for pectinase production in SSF by *Penicillium viridicatum*. Gayen and Ghosh (2011) used orange peel and wheat bran in ratio of 1:1 (w/w) for pectin methylesterase production in SSF. Heerd et al. (2012) also used citrus fruit waste along with wheat bran for polygalacturonase enzyme production using SSF (Heerd et al., 2012). Enhanced pectinase production was obtained

by Ruiz et al. (2012) using lemon peel pomace in a column-tray bioreactor operated at 30°C and 70% moisture for 96 h.

*Apple pomace*: Apple pomace, an apple processing industry waste presents a disposal problem. Dried apple pomace is rich in carbohydrates, pectin, and proteins. It has a pH range of 3.1–3.8 (Hang et al., 1982). The remaining 25%–35% of the fresh fruit weight after juice extraction is called apple pomace which contains 85% carbohydrate, 15% protein (Reid et al., 1999), and 12.3% fermentable sugar (Hang and Woodams, 1986). Apple pomace has both water-soluble components such as monosaccharide, oligosaccharide, water-soluble polysaccharide, and water-insoluble components-pectic substances. Berovic and Ostroversnik (1997) used 1500 g apple pomace along with 750 g of soya flour, 450 g of wheat bran, 1200 g of wheat corn, 90 g of dry whey, 15 g $(NH_4)_2SO_4$, and 60 g $NH_4NO_3$ in 2000 mL water at pH 7 for production of pectinolytic enzymes using *A. niger*.

Dried apple pomace has also been used as solid substrate for pectin methylesterase production by *A. niger* in SSF (Joshi et al., 2006).

*Strawberry pomace*: Chinese mushroom *Lentinus edodes* has been grown on strawberry pomace for the production of polygalacturonase (Zheng and Shetty, 2000). Maximum polygalacturonase production in SSF (29.4 U/g pomace) was obtained at 40 days growth of the mushroom on strawberry pomace as solid substrate.

*Grape pomace*: Washed grape pomace was supplemented with orange peel (1:1) and nutrient solution (g/L urea, 2.4; $(NH_4)_2SO_4$, 9.8; $KH_2PO_4$, 5.0; $FeSO_4{\cdot}7H_2O$, 0.001; $ZnSO_4{\cdot}7H_2O$, 0.0008; $MgSO_4{\cdot}7H_2O$, 0.004; $CuSO_4{\cdot}5H_2O$, 0.001 and pectin, 11.5) for the production of polygalacturonase, xylanase, and cellulase by SSF (Diaz et al., 2012). They reported increased enzyme production as compared to when whole grape pomace was used alone.

## 8.6.2 Production Time

The production time of pectinolytic enzymes varies with the nature of solid substrate and the selected microorganism. Maximum production time of pectic enzymes from different microorganisms varies from 1 to 7 days.

In Fig. 8.2A, the production time for endopolygalacturonase using papaya peel and orange peel individually by *A. niger* has been depicted, which shows the difference in production time with different substrates. Fig. 8.2B compares the endopolygalacturonase production with the change in fungal biomass measured as the glucosamine content. It is evident that the fungus hydrolyses the pectin rich substrate for its growth, producing pectin hydrolyzing enzymes in return.

A sharp increase in polygalacturonase production by *A. sojae* after the second day of incubation period was observed, giving maximum polygalacturonase production (136.9 U/gds) on the fourth day of

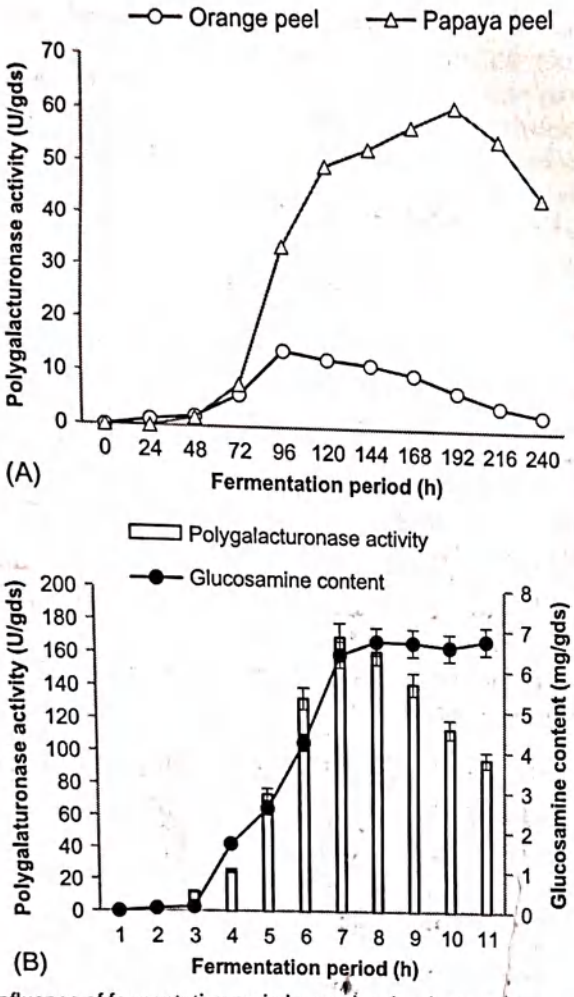


**Fig. 8.2** Influence of fermentation period on endopolygalacturonase production by *A. niger* utilizing (A) papaya peel and orange peel; and (B) combination of papaya peel and orange peel (2:1).

incubation using wheat bran as solid substrate (Demir and Tari, 2014). The production of polygalacturonase was shown to begin during the third and fourth day, utilizing the other carbon sources present in the media. Production of polygalacturonase on grape pomace using *A. awamori* strain was reported on the 25th hour of fermentation (Botella et al., 2005). Strawberry pomace was used as solid medium for production of polygalacturonase over a production period of 40 days (Zheng and Shetty, 2000). Diaz et al. (2012) showed that the addition of orange peels reduced the production time and increased the yield of the pectinolytic enzymes using grape pomace.

Patidar et al. (2016b) reported 120 h as optimum time for production of pectin methylesterase enzyme in SSF using *A. tubingensis* MP30. Joshi et al. (2006) reported 96-h production time for maximum pectin methylesterase production in SSF using apple pomace powder as solid substrate and *A. niger* as microbial source.

### 8.6.3 Production Temperature

Ambient temperature from 30°C to 37°C has been found to be optimum for fungal growth as well as the fungal pectinolytic enzyme production. Patil and Dayanand (2006), Hendges et al. (2011), and Demir and Tari (2014) obtained the maximum polygalacturonase production by *Aspergillus* strains at 34°C, 30°C, and 37°C, respectively. The influence of temperature is associated with the growth of the organism (Uzuner and Cekmecelioglu, 2015). Darah et al. (2013) reported highest fungal growth rate at 30°C production temperature. However, the production temperature and growth temperature can be different as reported by Demir and Tari (2014).

Joshi et al. (2006) reported 25°C production temperature for maximum pectin methylesterase production by *A. niger*. Similarly, Maldonado and Strasser de Saad (1998) and Patidar et al. (2016b) used 30°C production temperature for maximum pectin methylesterase production by *Aspergillus* sp.

### 8.6.4 Moisture Content and Moistening Agent

The industrial enzyme production using SSF is largely affected by the amount of moisture content in the media. A 40%–80% variation in initial moisture content has been used by various workers for pectinase production using different solid substrate and fungal strains. Heerd et al. (2014) used 0.2 N HCl to maintain 160% moisture content in wheat bran and sugar beet pulp (70:30) solid substrate. They also reported the use of 120% moisture content maintained by 0.2 N HCl in SSF containing wheat bran and orange peel (70:30) as solid substrate (Heerd et al., 2012).

Enhanced production of xylanase, polygalacturonase, and carboxymethyl cellulase using grape pomace and 70% moisture content maintained with nutrient solution was reported by Diaz et al. (2012) in wheat bran solid substrate. Demir and Tari (2014) reported use of 62% moisture content. They also reported 47% higher polygalacturonase production using distilled water over buffer solution pH 6.0. However, Ruiz et al. (2012) and Patidar et al. (2017) used 70% moisture content with Czapek-Dox medium and 90% moisture content with distilled water for maximum polygalacturonase production.

Pectin methylesterase production in SSF was also shown to be regulated by moisture content. Maldonado and Strasser de Saad (1998) showed 70% to be optimum whereas Gayen and Ghosh (2011) reported 50% and Patidar et al. (2016b) reported 86% moisture content to be optimum.

### 8.6.5 Particle Size

In SSF processes, particle size has been shown to influence aeration, specific area, and porosity of the substrate. Demir and Tari (2014) reported particle size of 150–250 μm as optimum for maximum polygalacturonase production. The sized particles of wheat bran gave 92% more of enzyme activity than the unsized particles. The optimum particle size for pomelo peel was reported to be 0.75 mm for maximum polygalacturonase production (Darah et al., 2013). The particle size range 0.7–2 mm (mesh size 25) of lemon peel pomace was reported by Ruiz et al. (2012) for maximum polygalacturonase production.

A particle size of 2-mm papaya peel was shown optimum for fungal growth and pectin methylesterase production by Patidar et al. (2016b). The SEM image of *A. tubingensis* MP30 growing over 2-mm papaya peel is shown in Fig. 8.1.

### 8.6.6 Inoculum of Microorganisms

Fungal inoculum is prepared using either a spore suspension or a vegetative seed culture prepared on a slant. The inoculum size is important in the SSF process. Taragano and Pilosof (1999), Silva et al. (2007), Tai et al. (2014) used spore suspension as inoculum for fungal enzyme production. However, Demir and Tari (2014, 2016) used seed culture inoculum for production of polygalacturonase. The spore suspension used for inoculation was prepared in distilled water, 0.1% Tween-80, and 0.2% Tween-80 by Taragano and Pilosof (1999), Silva et al. (2007), and Darah et al. (2013), respectively. Most commonly an inoculum size ranging from $10^5$ to $10^7$ spores/g of substrate is used (Silva et al., 2005; Botella et al., 2005; Hendges et al., 2011, Demir and Tari, 2016). *Bacillus subtilis* inoculum containing $10^6$ CFU/mL was incubated overnight under agitation at 130 rpm before inoculation by Uzuner and Cekmecelioglu (2015). Joshi et al. (2006) used *A. niger* spore suspension prepared in distilled water for production of pectin methylesterase enzyme in SSF. Patidar et al. (2016b) reported $1 \times 10^7$ spores per ml prepared in 0.1% Tween-80 as optimum for maximum pectin methylesterase production.

### 8.6.7 Agitation Frequency

Agitation is a major challenge for successful implementation of SSF (Favela-Torres et al., 2006). Agitation is required to remove the heat generated during metabolic process of fermentation and to mix the oxygen transferred to the solid medium. In SSF, the speed of agitation is reported to be once to thrice a day. Demir and Tari (2014) reported that agitation speed of 3 times per day increased the polygalacturonase production in SSF by 17.2% as compared to the static medium. Darah et al. (2013) on the other hand reported no agitation

requirement for maximum polygalacturonase production as the agitation of medium may affect the nature of particle and may damage fungal mycelia. Gayen and Ghosh (2011) reported maximum production of pectin methylesterase in stationary state using SSF.

## 8.7 Bioreactor Employed for Pectinolytic Enzyme Production Using SSF

The various important factors considered for the development of a SSF bioreactor include selection of microorganism and substrate, optimum process parameters to be used and methods for purification of the end product. Development of a fermentation process requires proper understanding of the relationships between the physiology of microorganism and fermentation parameters such as pH, temperature, moisture content, aeration, and nature of solid substrate employed.

Singhania et al. (2009) showed that the major challenges that led the researcher to thrive hard to find the solutions are scale up of SSF process and biomass estimation. A number of bioreactors have been designed to overcome the problems of scale up and online monitoring of parameters. These bioreactors also regulate the heat and mass transfer which are otherwise difficult to be managed in a basic system of fermentation. Several types of bioreactors have been designed for small scale as well as large-scale applications of SSF, including the tray bioreactors, packed-bed bioreactors, and drum bioreactors.

### 8.7.1 Tray Bioreactor

Tray bioreactor has been used for production of polygalacturonase, xylanase, and carboxy methyl cellulase (CMCase) in SSF by Diaz et al. (2013). Tray bioreactor was also used by Ruiz et al. (2012) for production of pectinase by *A. niger* Aa-20 using lemon peel pomace as support and carbon source. Tray reactors are less labor intensive, if run as a continuous system and they can also be easily scaled to larger operations. However, in this type of reactor, the temperature is difficult to control. Recently patents have been filed for novel designs of the tray reactor, in which a single-use fermentation tray is fitted into a rigid form, closed with an upper chamber and aeration is supplied between these two portions. In this design, temperature can be easily controlled (Roussos et al., 2014).

### 8.7.2 Packed-Bed Bioreactor

Packed-bed reactors provide larger substrate bed and easier product recovery. Singhania et al. (2009) suggested that the use of packed-bed reactor results in improper heat transfer over the larger substrate bed with a risk of drying out of the substrate as well as formation of air

channels. Diaz et al. (2013) used packed-bed reactor for production of polygalacturonase, xylanase, and CMCase.

### 8.7.3 Drum Bioreactor

Rodriguez-Fernandez et al. (2011) used horizontal drum bioreactor for pectinase production. In this reactor, $CO_2$ production is measured by an infrared sensor while consumption of $O_2$ by the microorganism is measured by two electrochemical sensors, one sensor fitted in the inlet and the other located in the outlet of the bioreactor. Drum bioreactors can perform constant mixing thereby ensuring thermal equilibrium, or intermittent stirring, whereby stationary phase is maintained. However, agglomerates may form and shear stress is increased over the intermittently stirred bioreactor which has a lower shear stress, but do not have effective aeration (Singhania et al. 2009).

### 8.7.4 Biomass Estimation

Separation of biomass is essential for the kinetic studies and is a big challenge in SSF. Estimation of oxygen intake and carbon dioxide evolution rate are considered to be most accurate for the determination of the growth of microorganism (Singhania et al., 2009). Glucosamine estimation, ergosterol estimation, protein (kjeldahl) estimation, DNA estimation, dry weight changes, and $CO_2$ evolution are indirect biomass estimation methods used in SSF (Rodríguez-Fernández et al., 2011; Ruiz et al., 2012). Nowadays, digital image processing developed for biomass estimation in SSF is used.

## 8.8 Industrial Applications of Pectic Enzymes

### 8.8.1 Fruit Beverage Industry

Pectic enzymes find various uses in the fruit and vegetable industries, fruit juice clarification, wine clarification, olive oil extraction, wood preservation, textile industries, etc. The enzymes necessarily act in one or more of the following ways:

1. reducing viscosity of concentrates;
2. removing cells from plant material increasing the yield of juice and solids; and
3. modifying and solubilizing pectic structures to affect sedimentation and clarification of juices.

Pectic polysaccharides act as suspending agents for pulp and other substances causing cloudiness and difficulty in filtration and clarification of juice concentrates. The pectic enzymes increase the efficiency of juice extraction, bringing about an increase in yield and reduction

in viscosity and destruction of suspending power, thereby increasing clarity and filterability. Pectinolytic enzymes are one of the upcoming enzymes of fruit industries. The role of acidic pectinases in bringing down the cloudiness and bitterness of fruit juices is well established (Kashyap et al., 2001; Jayani et al., 2005). In an unripe fruit, pectin is bound to cellulose microfibrils in the cell wall. Such pectin is insoluble and hence confers rigidity to cell walls. However, during ripening, the structure of pectin is altered by naturally occurring enzymes in the fruits. As a result of this, the pectin becomes more soluble and softens the plant tissues (Caffall and Mohnen 2009).

### 8.8.2 Fruit and Juice Processing

Fungal pectic enzymes are generally used to facilitate processing of fruits and are available commercially as pectinases under various trade names. Pectinex (trade name) manufactured by Swiss Ferment Co. Ltd., Switzerland is used for extraction and clarification of fruit juices and grape mast. Ciba-Geigy AG, Switzerland manufactures Ultrazyme 100 for the same purpose and Irgazyme M-10 for maceration of vegetables and fruits (Fogarty and Kelly, 1983). Production of a variety of sparkling clear juices and cloud juices depends on the pectic enzyme treatment (Rombouts and Pilnik, 1980; Whitaker, 1984; Voragen and Pilnik, 1989; Ismail et al., 2016; Sharma et al., 2017). The whole fruit along with peels and cores is crushed in a hammer mill and treated with pectinex or ultra SP-L at 100–200 g/ton for 30–60 min. It is then pressed, screened, and treated with pectinex 3XL at 100–200 g/1000 gallons in a clarification tank for 1–2 h at 54°C. The juice is then filtered and concentrated (Boyce, 1986). Nonenzymatic processes in the depectinization of fruit juices have also been used. However, the enzyme treatment increases the efficiency of pressing and juice extraction and even enhances flavor and color release, especially during continuous pressing operations (Whitaker, 1984; Voragen and Pilnik, 1989). Using both cellulases and pectinases, the cost of fruit processing is lowered because of the synergistic effect of these enzymes (Fogarty and Kelly, 1983). The synergistic effect is evident during liquefaction, as shown in case of apple pulp when the viscosity of the stirred pulp drops more rapidly during treatment with mixture of pectinase and cellulase enzyme preparations than either of them individually (Pilnik et al., 1975). Voragen et al. (1980) have studied the action of pure enzymes and their mixtures on pectin. The studies showed that pectin methylesterase and polygalacturonase individually release 0% and 21%, respectively of the pectic materials, and in combination release 75% of the pectic materials. Cellulase alone had little effect on pectin (5%) and solubilized only 22% of the cellulose. Combined cellulase-pectinase activities released 80% of the polysaccharides (Voragen et al., 1980).

The enzymatic treatment of the blackcurrants pulp before pressing improved the juice and color yield (Charley, 1969). Beltman and Pilnik (1971) have shown that enzymatic pectin degradation yielded thin free-run juice and a pulp with good pressing characteristics. High yields are always connected with enzyme treatment. Another advantage of the pulp enzyme process is that there is a better release of anthocyanins of colored fruits into the juice. This technique is used for red wines and is simpler than the classical fermentation "on the skin," which is generally used to obtain the desired color (Pilnik and Voragen 1993). Enzyme treatment of pulp of olives, palm fruit, and coconut to increase oil yield has also been described (Neubeck, 1975).

### 8.8.3 Fruit Juice Clarification

The oldest process for clarifying the fruit juices using the pectic enzymes still finds the largest market for these enzymes. The freshly pressed fruit juices especially, apple, pear, and grape juices are turbid and viscous. Treatment with pectinolytic enzymes rapidly reduces the viscosity and turbidity by settling out the cloudy particles by aggregation. The juice can be separated from these particles by filtration or centrifugation or simply siphoning out from the sediment. The end product so obtained is a sparkling clear juice (Li et al., 2017).

Careful experimentation with the purified enzymes has led to the conclusion that the clarification process is a combined effect of pectin methylesterase and polygalacturonase (Endo, 1965) or pectin lyase alone in case of apple juice, which contains highly esterified pectin (>80%) (Ishii and Yokotsuka, 1972). In grape juice, which contains pectin with a lower degree of esterification (44%–65%), pectin lyase alone does not perform as well (Ishii and Yokotsuka, 1973). Recently, Gainvors et al. (1994) have used *S. cerevisiae*, yeast, producing pectin degrading enzymes for the clarification of fruit juices. A magnetic tri-enzyme nanobiocatalyst comprising of amylase, pectinase, and cellulase has been used for clarification of grapes, apple, and pineapple juices (Sojitra et al., 2016). Pectin degrading enzymes are used to clarify following important fruit juices.

*Apple juice*: *Aspergillus* pectinolytic enzymes have been used for clarification of apple juice. A clearer apple juice with increased % transmission (1.7–5.6) was obtained after overnight treatment with pectin methylesterase and polygalacturonase (Joshi et al., 2011). Similarly, Kant et al. (2013) reported increase in % transmission from 1.7 to 20.4 upon overnight incubation with polygalacturonase enzyme. Sandri et al. (2013) reported a 90% decrease in apple juice turbidity using *A. niger* pectinase enzyme. Yuan et al. (2011) applied polygalacturonase of *Penicillium* sp. for apple juice which reduced the intrinsic viscosity of apple juice by 4.5%, and increased the light transmittance by

71.8%. *Bispora* sp. pectinase treated apple juice showed 84% increase in transmittance and reduction in viscosity by 7.7% (Yang et al., 2011). Effect of high pressure on apple juice clarification by pectin methylesterase has also been studied by Baron et al. (2006). Immobilized pectinase in reusable polymers has been used for clarification of apple juice (Rajdeo et al., 2016).

*Orange and mosambi juice*: Citrus fruits *viz.* oranges, lemon, and grapefruit are rich in pectin concentration (Galant et al., 2014). Commercially prepared citrus juices have one-third of the insoluble material as pectin, which is found within the juice cloud (Baker and Bruemmer, 1969). Orange pectin is partially methylated because of the removal of methoxyl group from pectin by pectin methylesterase (Kashyap et al., 2001; Maran et al., 2013). In orange juice, an undesirable precipitation of haze particles is formed due to the formation of calcium pectate in the presence of calcium ions. A mixture of pectinase, xylanase, and CMCase from *A. awamori* clarified orange juice by 95% in tray reactor (Diaz et al., 2013). Rai et al. (2004) obtained 89% clarified mosambi juice with *A. niger* pectinase using enzyme protein in a concentration 0.004 g/L for 99 min, at 42°C.

*Passion fruit juice*: This juice has commercial importance due to its pleasant unique aroma and flavor. Jiraratananon and Chanachai (1996) observed a reduction in viscosity by 18% with use of pectinase in passion fruit juice. Chitosan treatment was used with passion fruit juice for clarification by centrifugation at 4000 rpm followed by coagulation/flocculation process at 300 ppm and pH 6.

*Banana juice*: Pectin and starch are the main causes of turbidity of banana juice. Pectin makes the clarification process difficult because of its fiber-like molecular structure. The optimum conditions for clarification of banana juice are found to be 0.084% enzyme concentration, 43.2°C incubation temperature, and incubation time of 80 min by response surface methodology (Lee et al., 2006).

*Lemon juice*: *Penicillium occitanis* pectinase has been used for lemon juice clarification (Maktouf et al., 2014). The optimum treatment conditions reported were 600 U/L enzyme concentrations, 45 min and 30°C under optimized conditions with 77% reduction in viscosity, and 47% reduction in turbidity.

*Mango juice*: Pectinase from *A. foetidus* has been reported for mango juice clarification (Kumar et al. 2012). Mango juice was treated with 20 mL of crude enzyme preparation (specific activity 228 IU/mL). The maximum mango juice clarification (92.5%) was obtained at temperature of 40 C and 150 min incubation time.

*Pineapple juice*: Pectin methylesterase from *A. tubingensis* has been used for pineapple juice clarification (Patidar et al. 2016b). It was found that increase in amount of enzyme from 10 to 100 U increased the juice clarification from 3.1% to 19.5% and decreased the

pH from 4.3 to 3.0 at 30°C. Tochi et al. (2009) reported pineapple juice clarification by commercially available *A. niger* pectinase (Sigma-Aldrich) which showed reduction in turbidity from 1.5 to 0.8 at 35 C. de Carvalho et al. (2008) reported 5% change in pineapple juice sugar content due to addition of pectinase and cellulase followed by cross flow micro- and ultra-filtration to maintain the nutritional quality of pineapple juice.

*Blue berry juice*: Blueberry juice has nutritional potential and has been clarified by Sandri et al. (2013) using pectinase of *A. niger* produced in SSF.

*Guava juice*: South Africa, India, and Hawaii are major producers of Guava. Kant et al. (2013) used *A. niger* polygalacturonase for guava juice clarification. They showed that the addition of enzyme increased the mg% sugar content from 1.7 to 20.4 and % transmission at 650 nm from 1.9 to 4.8, simultaneously also reducing the pH of the juice.

*Pomegranate juice*: The juice upon treatment with pectinolytic and proteolytic enzymes underwent clarification and reduction in turbidity and haze (Cerreti et al., 2016, 2017). They have used response surface methodology for analysis of incubation time, temperature, and complex enzyme amount which was reported to be 100–110 min, 25–30 C, and 0.22–0.25 g%, respectively.

*Date syrup*: Dates play an important part in the economic and social lives of the people of the hot desert regions of the world. They are marketed globally as a high-value fruit (Abbès et al., 2011). The commercial quality of date syrup increases on addition of pectinase. The use of pectinase and cellulase enzyme (50U/5U) gave the highest recovery of total soluble solids and the lowest turbidity compared with control sample.

### 8.8.4 Maceration

Maceration is the process by which organized tissue is transformed into a suspension of intact cells, resulting in pulpy products used as base material for pulpy juices and nectars, as baby foods, and as ingredients for dairy products such as puddings and yoghurts (Pilnik and Voragen, 1993; Khatri et al., 2015).

Mechanical processes for manufacture of such products are not suitable because many cells are inevitably disrupted and the endogenous enzymes so released, damage flavor, color, and ascorbic acid. To limit the action of these enzymes, heat must be applied with the concomitant danger of damage. Enzymatic degradation of pectin after mild mechanical treatment improves product properties. Since the aim of the enzyme treatment is the transformation of tissue into a suspension of intact cells (Grampp, 1972; Bock et al., 1983), pectin degradation should affect only the middle lamella pectin. This process

is called enzymatic maceration. The so-called macerases are enzyme preparations with only polygalacturonase (Zetelaki-Horvath and Vas, 1980) or pectin lyase activities (Ishii and Yokotsuka, 1975). For vegetable maceration, bacterial endopectate lyase is preferred due to its alkaline optimum pH (Rombouts et al., 1978; Bock et al., 1983).

### 8.8.5 Wine Making

Pectic enzymes are also useful in wine making from grapes and other fruits including berries, peaches, apples, pears, etc. (Fogarty and Keliy, 1983; Villettaz, 1984; Bigelis, 1993). Pectic enzymes are used at various stages of the wine-making process, mainly crushing of fruits, before fermentation and after fermentation. During the crushing of fruits, addition of pectic enzymes increases the volume of free run juice and reduces the pressing time. It also aids in juice filtration and must clarification. The treatment with pectic enzymes improves the color yield due to extraction of pigments. Treatment of the juice with pectic enzymes before or during fermentation, settles out many suspended particles along with some undesirable microorganisms. The result is clearer wine and firmer yeast sediment. Addition of pectic enzymes to the fermented wine increases filtration rate and clarity. However, the amount of enzyme has to be adjusted taking into account the inhibitory effect of alcohol on pectinases. Addition of pectic enzymes after fermentation promotes flocculation and precipitation of pectin particles, floating microorganisms, and protein. Elimination of protein improves stability of wine. The use of pectic enzymes during all the three stages of wine making promotes a faster aging of the wine (Bigelis, 1993).

### 8.8.6 Coffee, Cocoa, Tea, and Tobacco Fermentation

The processing of coffee depends on fermentation with pectinolytic microorganisms to remove the mucilage coat from the coffee beans. A diluted commercial enzyme preparation is sprayed onto the cherries at 2–10 g/ton at 15–20 C. The fermentation stage of coffee processing is accelerated by the enzymatic treatment and is reduced from 40 to 80 h to about 20 h (Bigelis, 1993). Similarly in case of cocoa seeds encased in a mass of white mucilaginous pulp, pectic enzymes are applied to liquefy the mucilaginous mass. Pectinase treatment also accelerates tea fermentation at a carefully adjusted dose. Addition of pectinase also improves foam forming property of instant tea powder by destroying tea pectins (Willson and Clifford, 1992). During tobacco fermentation, the pectic constituent of the tobacco

leaves undergoes changes. Tobacco leaves are rich source of pectin methylesterase and during fermentation, the enzyme acts on pectinic acid in tobacco leaves, causing formation of free methanol. Procedure for the production of tobacco extracts using pectolytic enzymes has also been patented (1994).

Apart from the above applications, pectic enzymes have also been used in analytical work, in protoplast isolation, retting of textile fibers, preparation of galacturonic acid (Sakai et al., 1993), and unicellular foods (Whitaker, 1984). Pectin methylesterase has been used in stabilization of cloud in orange juice. Pectic enzymes are also involved in plant diseases, especially during pathogenesis and fruit rots.

## 8.9 Conclusion

The fruit juice beverage industry in particular uses pectic enzymes for enhanced juice extraction and its clarification. The pectinases used in fruit industry are mostly produced from fungal sources by SSF using various production systems and solid medium, mostly fruit waste. The fruit and agricultural waste produced annually worldwide is mostly used as an eco-friendly and economic substrate for pectinase production. Various fungi have been used for production of pectic enzymes using SSF bioreactors by several researchers for application to fruit juice clarification and enhancement of yield from fruit pulp. Also, it has been noted that the enzyme preparation used in fruit industries is generally a mixture of enzymes rather than a purified enzyme. The studies till date have described various bioreactors for pectinase production. This chapter is an attempt to describe the different types of pectic enzymes, their sources and production of hydrolytic enzymes suggesting their applications in fruit beverage industry. It has been observed that the methods applied for sustainable production of pectinase enzyme and their industrial applications are varied depending on the fungal sources and the choice of the beverage industry. Uniformity in the industrial application is thus desired. An interdisciplinary approach in production of enzyme and its application in fruit juice beverage industry to enhance the product quality can be sought.

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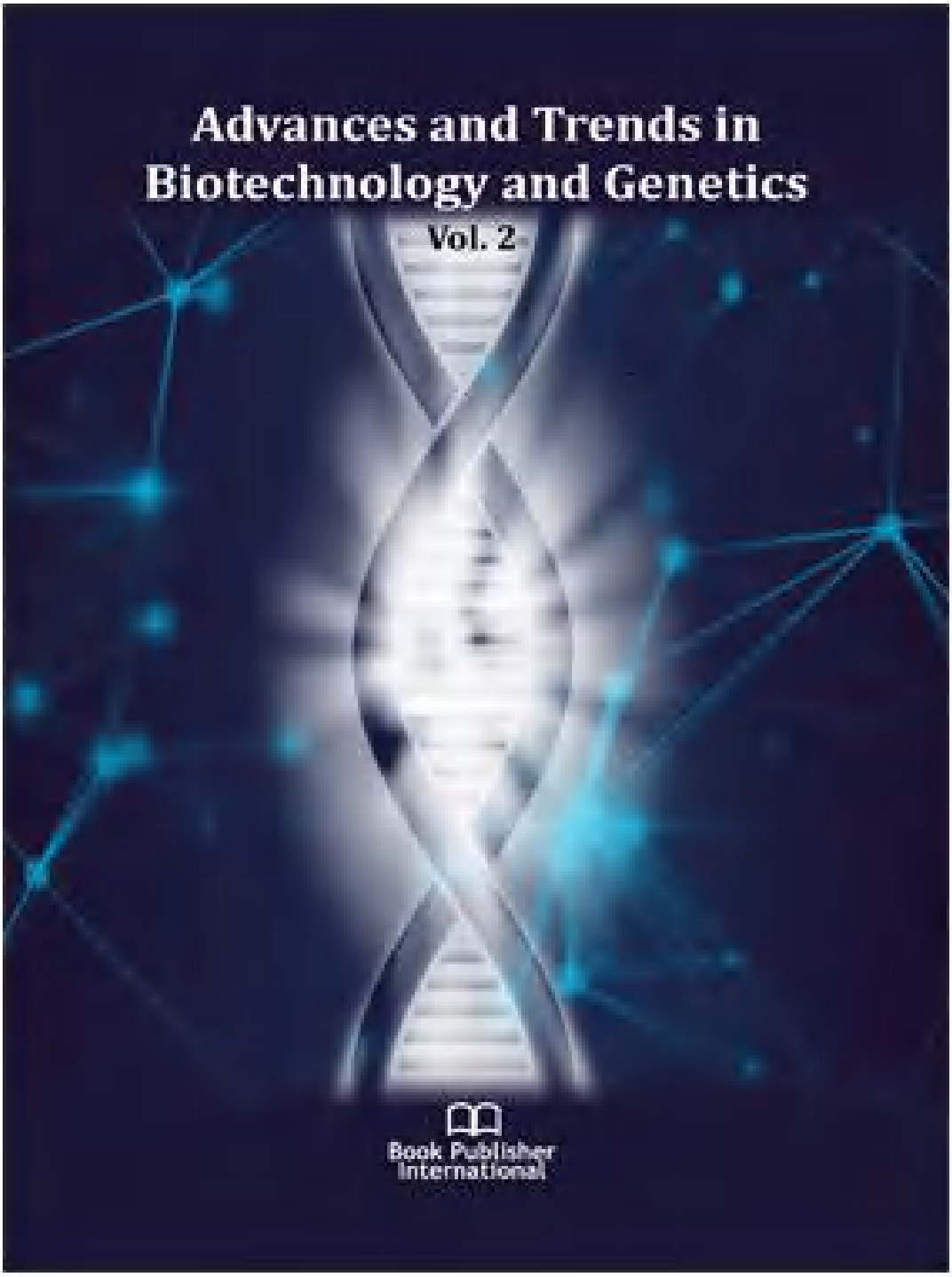
Advances and Trends in
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# Advances and Trends in Biotechnology and Genetics Vol. 2

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## Synopsis

*This book covers all areas of biotechnology, genetics and other related fields. The contributions by the authors include pectin methylesterase, ion exchange chromatography, gel filtration chromatography, solid state fermentation, Streptomyces, enzyme, renal-toxicity, nicotine, endo-1,4-β-xylanase, birchwood xylan, animal feedstocks, xylooligosaccharides, vitamin, chronic exposure, microarrays, RNA-seq, Background Subtraction (BS), Differentially Expressed Genes (DEGs), diabetes mellitus, β-cell of pancreas, stem cell therapy, pluripotent stem cells, hyperthermophilic, alkaline, methaemoglobin, carboxyhaemoglobin, spectrophotometer, pregnant women and hepatitis B, malaria, artesunate-amodiaquine, artemether-lumefantrine, pfK13-propeller, PfK13A369G, Cold Shock Proteins (CSP), Cold Inducible RNA-binding Protein (CIRP), RNA Binding Motif 3 (RBM3) etc. This book contains various materials suitable for students, researchers and academicians in the field of biotechnology and genetics.*


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# Purification and Characterization of Pectin Methylesterase Produced in Solid State Fermentation by Aspergillus tubingensis | Chapter 03 | Advances and Trends in Biotechnology and Genetics Vol. 2

*Aim:* *Purification and characterization of pectin methylesterase produced by Aspergillus tubingensis in solid state fermentation.*

***Study Design:*** *Pectin methylesterase enzyme produced by A. tubingensis was extracted from the fermented solid medium and purified using chromatographic techniques. The purified enzyme was characterized for physico-chemical and kinetic properties.*

***Place and Duration of Study:*** *Experiments were performed at the School of Biotechnology, Devi Ahilya University, Indore, INDIA and Maharaja Ranjit Singh College of Professional Sciences, Indore, INDIA, between October, 2014 and August, 2015.*

***Methodology:*** *The enzyme was extracted and purified using ammonium sulphate fractionation, ion exchange chromatography (IEC) using CM- cellulose and gel filtration chromatography (GFC) using Sephadex G-100. The molecular weight of the purified enzyme was determined using native polyacrylamide gel electrophoresis (Native PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). The purified enzyme was characterized to determine the pH and temperature optima. Thermostability, pH stability and substrate kinetics were studied for purified pectin methylesterase.*

***Results:*** *The acidic pectin methylesterase of Aspergillus tubingensis was purified to 20.3 fold with a 47.7% recovery through IEC on CM- cellulose and GFC using Sephadex G-100. The purified enzyme had a specific activity, 112.6 U/mg. The SDS-PAGE revealed that the enzyme was monomeric with a molecular weight of 45.7 kDa. The optimum pH and temperature were 4.6 and 50°C, respectively. This enzyme was stable over a wide pH range (3.0–8.0) and at relatively high temperature at 50°C for 1 h. The Km and Vmax values of pectin methylesterase towards citrus pectin were 33.3 mg/l and 251.2 µmol/ml/min, respectively. In addition, the enzyme activity increased by about 16% in the presence of 5 mM $Mg^{2+}$.*

***Conclusion:*** *The pectin methylesterase enzyme of A. tubingensis has been purified up to homogeneity and found to be monomeric on SDS-PAGE. Enzyme characterization revealed that purified enzyme worked optimally in acidic conditions and was stable at wider pH range.*

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# Optimal Reactive Power Dispatch for Enhancement of Static Voltage Stability using Jaya Algorithm

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***Abstract*— This paper presents a new Jaya optimization algorithm to minimize transmission line loss by using optimal reactive power dispatch (RPD). Transmission line loss have been evaluted and simultaneously maximizing reactive reserve accounting voltage stability inequality and equality constraints using continuation power flow methodology. Schur's inequality is selected as a proximity indicator which indicates the state of ...ge stability. The proposed Jaya optimization algorithm has been implemented on standard IEEE 30-bus test system. Performance of the Jaya algorithm has been compared with Shuffled Frog Leaping Algorithm (SFLA) and Particle Swarm Optimization (PSO). The obtained results show that the proposed Jaya algorithm is more effective, better convergence characteristics and robustness as compared to SFLA & PSO algorithms.**

***Keywords—Static voltage stability; Reactive reserve; Schur's inequality; Optimal reactive power dispatch; Transmission losses; Jaya algorithm.***

## I. INTRODUCTION

The optimal reactive power dispatch (ORPD) problem has been one of the most important aspects in power system planning & operation. The ORPD is a non-linear, non-convex & non-differentiable optimization problem. It is used to minimize line loss, maximize of reactive power reserve, improve voltage magnitude & enhance voltage stability by adjusting reactive power control variables such as PV- bus ...ages, tap settings of the transformer, and reactive power output of shunt VAR compensators in a power system while satisfying several equality and inequality constraints. There are many classical approaches that are used to solve OPRD problem such as Newton method, linear programming (LP), non-linear programming (NLP), quadratic programming (QP), (DQP), gradient search (GS) method, gradient projection method, reduced gradient method, interior point method and modified interior point method [1, 2]. These methods are computationally fast, and convergence with the fitness function of continuous, differentiable and single peak value but these methods can not optimize non-linear, discrete–continuous fitness function. The ORPD is a non-linear multimodal optimization problem with a mixture of discrete and continuous variables. It has multiple local optima. Hence, it is so difficult to find the global optimum solution of reactive power dispatch problem by using classical approaches. For these reasons, researchers have developed computational intelligence-based optimization techniques to solve the optimal reactive power dispatch problem. In recent years, computational intelligence-based techniques, such as multi-agent-based particle swarm optimization (MBPSO) [3], particle swarm optimization (PSO), Co-ordinated aggregation based particle swarm optimization (CAPSO), comprehensive learning particle swarm optimization (CLPSO) [4, 5], teaching learning based optimization (TLBO), genetic algorithm (GA), self adaptive differential evolution (SaDE), ensemble of mutation and crossover strategies and parameters in differential evolution (EPSDE) [6], biogeography-based optimization (BBO) [7], opposition-based gravitational search algorithm (OBGSA) [8], gravitational search algorithm (GSA) [9], seeker optimization algorithm (SOA) [10], Nelder–Mead simplex search (NMSS), hybrid shuffled frog leaping algorithm (HSFLA) [11], ANN and fuzzy based techniques have been proposed for solving ORPD problem. Basu [12] presented a multi-objective differential evolution (MODE) to minimizing transmission line loss, voltage deviation and enhanced voltage stability by using reactive control variables. Rajan and Malakar [13] proposed a method to solving optimal reactive power dispatch (ORPD) problem using hybrid Nelder–Mead simplex based firefly algorithm. Bhattacharyya & Saurav [14] developed a reactive power planning by using PSO based bio inspired algorithm.

This paper proposes a novel Jaya algorithm for solving optimal reactive power dispatch (ORPD) problem by adjusting the on-load transformer tap setting (OLTC) and shunt VAR compensations in order to achieve minimum transmission line loss, maximum reactive power reserve, improved voltage profile and enhanced voltage stability. Schur's inequality has been used as proximity indicator [15]. Proximity indicator has large value in the stable region of PV-curve and nearly equal to zero, at the collapse point. Hence, the magnitude of Schur's inequality is an indicator of relative voltage stability. A threshold value of this indicator can be assumed on the basis of experience for a specific system [16]. Part-2 gives problem formulation. Part-3 shows execution of the developed Jaya optimization algorithm. Part-4 shows results and discussions. Part-5 gives conclusions and highlights of the paper.

IEEE Bombay Section/Madhya Pradesh Sub-Section

## II. PROBLEM FORMULATION

The fitness function is to be selected as minimization of the total line loss (active & reactive power loss).

$$F = \sum_{i=1}^{NL} S_{loss,l} \quad (1)$$

$$S_{loss} = S_{gt} - S_{dt}$$

NL - Number of transmission line.

$S_{loss}$ - Line loss.

$S_{gt} = (P_{gt} + i * Q_{gt})$ – Total power generation

$S_{dt} = (P_{dt} + i * Q_{dt})$ – Total power demand

The reactive power reserve ($Q_{gi(res)}$) of the $i^{th}$ generator buses:

$$Q_{gi(res)} = Q_{gi}^{max} - Q_{gi} \quad (2)$$

The transmission line loss can be optimized by proper controlling of different control variables denoted by vector U.

$$U = [Vg_1, \dots, Vg_{NG}, Q_{C1}, \dots, Q_{CNC}, Tap_1, \dots, Tap_{NT}] \quad (3)$$

Vg - Voltage of the generator bus.

$Q_C$ - Shunt capacitor of the load bus.

Tap – Tap changing transformer.

NG - Number of generator buses.

NC - Number of VAR Compensators.

NT - Number of tap changing transformer.

Above fitness function is optimized subject to following equality and inequality constraints.

(i) Power flow equations equality constraint:

$$P_{gi} - P_{di} - V_i \sum_{j=1}^{NB} V_j [G_{ij} \cos(\delta_i - \delta_j) + B_{ij} \sin(\delta_i - \delta_j)] = 0$$

$$Q_{gi} - Q_{di} - V_i \sum_{j=1}^{NB} V_j [G_{ij} \sin(\delta_i - \delta_j) - B_{ij} \cos(\delta_i - \delta_j)] = 0$$

$$i = 1,2,3, \dots, NB \quad (4)$$

NB - Number of buses.

$P_{gi}, Q_{gi}$ - Active & reactive power generation at $i^{th}$ buses.

$P_{gi}, Q_{gi}$ – Active & reactive power demand at $i^{th}$ buses.

$V_i, V_j$ - Voltage magnitude of $i^{th}$ & $j^{th}$ buses.

$\delta_i, \delta_j$ – Phase angle of voltages of the $i^{th}$ & $j^{th}$ buses.

$G_{ij}, B_{ij}$ - Transfer conductance & susceptance between $i^{th}$ & $j^{th}$ buses.

(ii) Reactive power generation inequality constraint:

$$Q_{gi}^{min} \le Q_{gi} \le Q_{gi}^{max} \quad (5)$$

$$i = 1,2,3, \dots, NG$$

$Q_{gi}^{min}$ and $Q_{gi}^{max}$ - Minimum & maximum limits of reactive power generator at $i^{th}$ buses.

(iii) Shunt VAR compensator inequality constraints:

$$Q_{ci}^{min} \le Q_{ci} \le Q_{ci}^{max} \quad (6)$$

$$i = 1,2,3, \dots, NC$$

$Q_{ci}^{min}$ and $Q_{ci}^{max}$ - Lower and upper limit of reactive power injection of the $i^{th}$ shunt compensator.

(iv) Transformer inequality constraints:

$$Tap_i^{min} \le Tap_i \le Tap_i^{max} \quad (7)$$

$$i = 1,2,3, \dots, NT$$

$Tap_i^{min}$ and $Tap_i^{max}$ - Lower and upper limit of tap setting of the transformer $i^{th}$ transmission line.

(v) Load bus voltage magnitude inequality constraints:

$$V_i^{min} \le V_i \le V_i^{max} \quad (8)$$

$$i = NG + 1, \dots, NB$$

$V_i^{min}$ and $V_i^{max}$ - Lower and upper limit of voltage of the $i^{th}$ load buses.

(vi) Proximity indicator Inequality constraint:

$$\tau \ge \tau^{th} \quad (9)$$

$\tau$ - Proximity indicator (Schur's inequality)

$\tau^{th}$-Threshold value proximity indicator (Schur' s inequality)

## III. IMPLEMENTATION OF JAYA ALGORITHM TO SOLVE FORMULATED PROBLEM [17]

**Step 1:** Data input: Control variables and system parameters (resistance, reactance, and susceptance etc.)

**Step 2:** Evaluate load flow solution under base case using continuation power flow methodology.

**Step 3:** Initialization; Initialize the population of size 'M' for reactive power control variables (PV-bus voltage magnitudes, OLTC and VAR compensators). Each individual $U_t$ is a vector that contains as many control variables D. Generated population is randomly distributed in the range $[U_{ij}^{min} \le U_{ij} \le U_{ij}^{max}]$, $j = 1,2,3, \dots, D$

$$U_i^0 = [u_{i1}^0, u_{i2}^0, u_{i3}^0, \dots \dots, u_{iD}^0]^T \quad (10)$$

$$i = 1,2,3, \dots, M$$

**Step 4:** Calculate fitness function for the feasible vectors and rank the population according to their respective minimum value of fitness function.

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tep 5: Based on the value of fitness function, identify the best candidate ($U_{best}$) and worst candidate ($U_{worst}$).

tep 6: Set generation count k = 1.

tep 7: Update the population based on best candidate ($U_{best}$) and worst candidate ($U_{worst}$).

$$U_i^{(k+1)} = U_i^k + rand_1 * (U_{i,best}^k - |U_i^k|) - rand_2 * (U_{i,worst}^k - |U_i^k|) \quad (11)$$

$U_{i,best}^k$ - is the best candidate value of the $k^{th}$ variables.

$U_{i,worst}^k$ - is the worst candidate value of the $k^{th}$ variables.

$U_i^{(k+1)}$ & $U_i^{(k)}$- is the candidate value at generations $k^{th}$ & $(k+1)^{th}$ respectively.

$rand_1$ & $rand_2$- is the two random numbers [0, 1].

$rand_1 * (U_{i,best}^k - |U_i^k|)$ – is the solution to move closer to the best solution.

$rand_2 * (U_{i,worst}^k - |U_i^k|)$ – is the solution to avoid the st solution.

**Step 8:** Select $U_{i,new}^{(k+1)}$ in new population or reject it to retain $U_i^{(k)}$ in new population.

$$U_{i,new}^{(k+1)} = \begin{cases} U_i^{(k+1)}, & \text{if } [f(U_i^{(k+1)}) \le f(U_i^{(k)})] \\ U_i^{(k)}, & \text{Otherwise} \end{cases} \quad (12)$$

**Step 9:** Run continuation power flow program incorporating updated $U_{new,i}$. If updated $U_{new,i}$ minimize fitness function go to next step. Otherwise go to step 5.

**Step 10:** Increase generation count $k = k + 1$. If $k \le k_{max}$ repeat from step 5. Otherwise stop.

## IV. Results & Discussions

In this, Jaya algorithm has been applied on IEEE 30-bus standard test systems for the solution of optimal reactive power dispatch (ORPD) problem. The proposed algorithm is implemented using the MATLAB R2008a software and run on PC with Intel (R) Core(TM) i3-3120M CPU @ 2.50 GHz .00 GB RAM. Developed algorithms have been implemented for minimizations of transmission line loss and maximization of reactive power reserve at generator buses.

### IEEE 30-Bus System

The 30-bus system consists of 6 generators, 24 load buses & 41 transmission lines. This system has 12 reactive power control variables; which are 6 generators (bus no. $1^{st}$, $2^{nd}$, $5^{th}$, $8^{th}$, $11^{th}$ & $13^{th}$), and 4 OLTC (line number $11^{th}$, $12^{th}$, $15^{th}$ & $36^{th}$) and 2 shunt compensations are connected at buses $10^{th}$ and $24^{th}$, The limits of generators bus voltages and OLTCs have been assumed as 0.95pu to 1.15pu, and 0.90 to 1.10 respectively. Shunt compensations limit (lower and upper) of bus no. $10^{th}$, 0.00pu to 0.19pu and bus no. $24^{th}$, 0.00pu to 0.04pu [18] respectively. Reactive power limits (minimum and maximum) of generating bus no. $1^{st}$ lying between −0.2000pu to 1.5000pu, bus no. $2^{nd}$ lying between −0.2000pu to 0.6000pu, bus no. $5^{th}$ lying between −0.1500pu to 0.6250pu, bus no. $8^{th}$ lying between −0.1500pu to 0.5000pu, bus no. $11^{th}$ lying between −0.1500pu to 0.4000pu, bus no. $13^{th}$ lying between −0.1500pu to 0.4500pu [18]. The desired range of load bus voltage is 0.95pu to 1.05pu. The total base case active and reactive power demand on the system are 4.2199pu & 1.8906pu, value of proximity indicator ($\tau$) = 0.1634 and fitness function (F) = 0.8176pu. From operational experience of system threshold value of proximity indicator is selected as $\tau^{th}$ = 0.1750 [16]. Initially, 20 populations of each control variable have been generated randomly using excel software according distribution characteristic of control variable. Maximum numbers of generation is taken as 500 and terminated after 298 generations that are no improvement in fitness function. Table - 1 shows the comparison of each algorithm to find the best optimal control variable settings with and without optimization using Jaya, SFLA and PSO techniques [19, 20]. Table - 2 shows the comparison of most sensitive load bus voltage magnitude with and without optimization using Jaya, SFLA and PSO techniques. Table - 3 shows the comparison of Jaya algorithm with SFLA and PSO techniques based on of proximity indicator, total reactive power reserve, static stability limit and fitness function (total transmission line losses). The total transmission loss and reactive power reserve obtained by the proposed Jaya algorithm is 0.7068pu & 1.7298pu respectively, which is best among all other methods. It is observed that Jaya algorithm is able to reduce the total transmission loss by 13.55% with respect to the base case solution (initial loss), against 12.16% with SFLA and 10.40% with PSO [13]. "Fig. 4," shows a plot for comparison of the convergence of fitness function with respect to number of generation for Jaya, SFLA & PSO techniques.

## V. Conclusion

This paper present a new Jaya based optimization algorithm for solving highly nonlinear non-convex optimal reactive power dispatch (ORPD) problem with equality constraints of power system. In this paper, minimization of transmission line losses, maximization of reactive power reserve and enhancement of voltage profile for accounting operating & stability inequality constraints are considered. These objectives are achieved by the rescheduling of the reactive control variables such as; generator voltages magnitude, OLTC and static VAR compensations. Jaya optimization algorithm is based on the concept that the result obtained for a given problem should avoid the worst result and travel towards the best result. This algorithm requires only the common control parameters and does not require any algorithm-specific control parameters. This optimization algorithm is an efficient optimization method for large scale non-linear optimization problems for finding the global solutions. Simulation results obviously demonstrate the

oposed Jaya algorithm is able to produce better result ompared to other optimization techniques for IEEE 30-bus test systems. Main contributions of this paper are:

(i) Determination of transmission line losses.

(ii) Determination of reactive power reserve.

(iii) Development of an algorithm for maximization of reactive power reserves in order to maintain voltage profile for accounting operating & stability inequality constraints using Jaya algorithm.

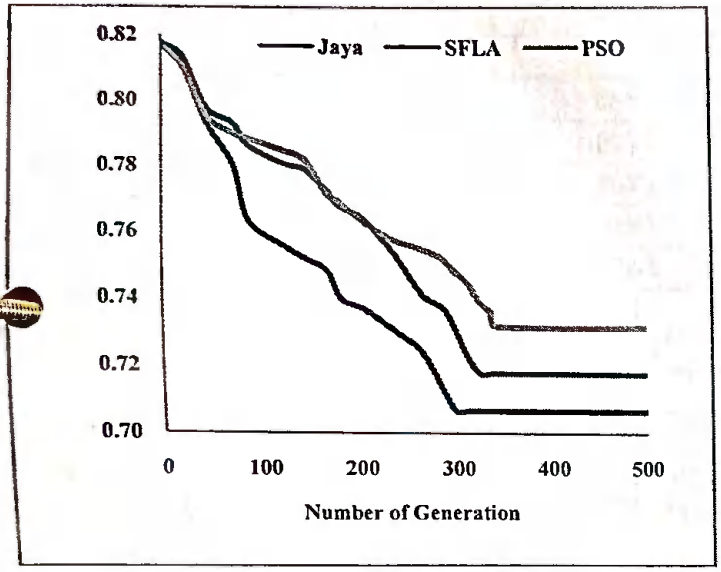


Fig. 4. Plot of convergence of fitness function with respect to number of generation using Jaya, SFLA & PSO algorithms for IEEE 30-bus system.

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**Table - 1. Reactive power control variables using Jaya, SFLA and PSO algorithms for IEEE 30-bus system $(S_{dt}) = 4.6241pu$.**

| Sr. No. | Reactive Control Variables | Methodology | | | |
|---|---|---|---|---|---|
| | | Base Case | Jaya | SFLA | PSO |
| 1 | $V_{g1}$ | 1.0851 | 1.0849 | 1.0842 | 1.0843 |
| 2 | $V_{g2}$ | 1.0585 | 1.0583 | 1.0576 | 1.0476 |
| 3 | $V_{g5}$ | 1.0321 | 1.0219 | 1.0212 | 1.0112 |
| 4 | $V_{g8}$ | 1.0471 | 1.0269 | 1.0262 | 1.0162 |
| 5 | $V_{g11}$ | 1.0854 | 1.0852 | 1.0845 | 1.0847 |
| 6 | $V_{g13}$ | 1.0937 | 1.0935 | 1.0828 | 1.0928 |
| 7 | $Tap_{11}$ | 1.0685 | 0.9342 | 0.9424 | 0.9346 |
| 8 | $Tap_{12}$ | 1.0693 | 1.0695 | 1.0692 | 1.0694 |
| 9 | $Tap_{15}$ | 1.0762 | 0.9464 | 0.9598 | 0.9095 |
| 10 | $Tap_{36}$ | 0.9216 | 1.0716 | 1.0865 | 1.0882 |
| 11 | $Qc_{10}$ | 0.0105 | 0.175 | 0.1771 | 0.1758 |
| 12 | $Qc_{24}$ | 0.0036 | 0.0396 | 0.0146 | 0.0308 |

**Table - 2. Most sensitive load bus voltage magnitude using Jaya, TLBO, DE and CAPSO algorithms for IEEE 30-bus system $(S_{dt}) = 4.6241pu$..**

| Sr. No. | Most Sensitive Load Bus | Base Case | JAYA | SFLA | PSO |
|---|---|---|---|---|---|
| 1 | $V_{17}$ | 0.8980 | 0.9960 | 0.9873 | 0.9951 |
| 2 | $V_{18}$ | 0.8782 | 0.9770 | 0.9667 | 0.9791 |
| 3 | $V_{19}$ | 0.8688 | 0.9695 | 0.9598 | 0.9701 |
| 4 | $V_{20}$ | 0.8762 | 0.9767 | 0.9674 | 0.9765 |
| 5 | $V_{21}$ | 0.8797 | 0.9828 | 0.9743 | 0.9805 |
| 6 | $V_{22}$ | 0.8815 | 0.9849 | 0.9764 | 0.9827 |
| 7 | $V_{23}$ | 0.8797 | 0.9823 | 0.9715 | 0.9844 |
| 8 | $V_{24}$ | 0.8631 | 0.9749 | 0.9649 | 0.9738 |
| 9 | $V_{25}$ | 0.8723 | 0.9912 | 0.9884 | 0.9909 |
| 10 | $V_{26}$ | 0.8513 | 0.9728 | 0.9700 | 0.9726 |
| 11 | $V_{27}$ | 0.8884 | 1.0103 | 1.0121 | 1.0107 |
| 12 | $V_{29}$ | 0.8429 | 0.9716 | 0.9734 | 0.9719 |
| 13 | $V_{30}$ | 0.8178 | 0.9502 | 0.9521 | 0.9505 |

able - 3. **Comparison of Jaya algorithm with SFLA and PSO techniques based on proximity indicator, total reactive power reserve, static stability limit and fitness function for IEEE 30-bus test system**

| Sr. No. | Methodology | Proximity Indicator (Schur's inequality) | Total Reactive Power Reserve (pu) | Fitness Function (pu) | Stability Limit (pu) |
|---|---|---|---|---|---|
| 1 | **JAYA** | **0.1790** | **1.7298** | **0.7068** | **7.3538** |
| 2 | SFLA | 0.1779 | 1.6934 | 0.7182 | 6.9442 |
| 3 | PSO | 0.1772 | 1.686 | 0.7326 | 6.5346 |
| 4 | Base Case | 0.1634 | 1.4172 | 0.8176 | 5.9203 |

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# Enhancement of Voltage Security by MW-Generation Rescheduling based on Sensitivities using Black Hole

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***Abstract*—This paper presents a new methodology for improving voltage stability under stressed condition by active power rescheduling accounting inequality constraints. Whenever the reactive power control variables are exhausted, then only active power control variables are used to enhance the voltage profile as well as to obtain desired voltage stability margin. MW-generation rescheduling using the algorithm increases considerable voltage security margin and load bus voltage as well. The objective is to optimize minimum eigenvalue of load flow Jacobian which indicates the proximity indicator of present operating point to saddle node bifurcation point. The minimum eigenvalue of load flow Jacobian has been optimized using Black Hole (BH) algorithm. Proposed methodology has been implemented on IEEE 14-bus test system. Performance of the developed algorithm are compared based on statistical inference of the fitness function with, Teaching Learning Based Optimization (TLBO) and Particle Swarm Optimization (PSO) techniques. The simulation results are good enough to accept and validate the proposed methodology.**

***Keywords—MW-generation rescheduling; BH; Minimum eigenvalue; Static voltage stability.***

## I. INTRODUCTION

Power systems are being operated closer to their stability limits due to economic and environmental constraints. Voltage stability has become an increasingly important factor in the operation and planning of electric power systems. At any point of time, the power system operating condition should be stable; meeting various operational criteria and it should also be secure in the event of any credible contingencies. There are different measures against voltage instability in real time and in the planning and designing stage of a power system.

Reactive power management from voltage stability viewpoint has been an important issue in recent years. Enhancement in static voltage stability limit is usually achieved by rescheduling of reactive power control variables. Since power, networks are heavily loaded and operate very near to voltage collapse point. Moreover, reactive power resources, most of the times touch the limits. There are situations where voltage security enhancement by reactive power control variables may not be feasible. In such situation, voltage stability margin can be enhanced by rescheduling of MW-generation, if such possibility exists owing to strong coupling between active power and voltage under stressed condition, under outage condition or due to excessive MW transfer along a transmission line, which may be dictated due to economic considerations [1]. Mansour et al. [2] used modal analysis for optimum location of static VAR compensation. A methodology for voltage stability improvement developed by Verma et al. [3] by controlling PV bus voltages and minimizing reactive power loss of the network. Mousavi et al. [4] presented a method of enhanced voltage stability margin by using reactive power management. Dike and Mahajan [5] presented voltage stability index-based reactive power compensation. Basu [6] presented a minimization of active power, voltage deviation and maximizing voltage stability by using reactive control variables. Titare et al. [7] developed an optimal reactive power rescheduling algorithm to enhance static voltage stability. All the research papers for voltage stability enhancement cited above use reactive power control variables for optimization. There may be situations where considerable voltage stability enhancement could be achieved by chosen MW-participations. Kirchen and Meeteren [8] have attempted to control the voltage within limit by MW-generation rescheduling. However, voltage magnitude itself is a poor indicator of proximity to system collapse condition. Under emergency condition, the MVA distance to voltage collapse is of significance instead of voltage magnitude. Srivastava and Srivastava [9] presented the effect of generation rescheduling to enhance the voltage stability margin. Chung et al. [10] described a strategy to improve the power transfer capability constrained by small-signal stability by appropriate active power generation rescheduling based on sensitivity. Dutta and Singh [11] presented a technique for optimal generators rescheduling technique for congestion management based on sensitivities of power flow through congested line. Venkaiah and Vinod Kumar [12] presented a new methodology for static congestion management (CM) by optimal rescheduling of active powers of generators. The selection of generator buses was based on the sensitivity of the congested line. Proximity indicator becomes important in such situation. Singh et al. [13] presented new algorithm for active power generation rescheduling at selected generator.

This paper developed, a new methodology for optimizing the proximity indicator with respect to MW-generations will improve voltage stability margin. Minimum eigenvalue of load flow Jacobian has been used as a proximity indicator as well as objective function. As the system is stressed minimum

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eigenvalue continuously decreases and becomes zero at collapse point. Larger the value of minimum eigenvalue at operating point, larger its distance from voltage collapse point. The objective of this paper is to develop an algorithm for increasing the voltage stability margin by maximizing the value of proximity indicator at current operating point using BH algorithm. Section-2 describes an evaluation of minimum eigenvalue of load flow Jacobian. Section-3 presents problem formulation. Section-4 presents solution of problem using developed algorithms BH for optimizing the fitness function. Section-5 gives results and discussions. Section-6 presents conclusions and highlights of the paper.

## II. MINIMUM EIGENVALUE OF LOAD FLOW JACOBIAN

Minimum eigenvalue of load flow Jacobian is one of the important proximity indicators for the assessment of voltage stability of a given system. The value of this indicator is inversely proportional to the system load. Minimum eigenvalue of load flow Jacobian is evaluated as follows:

$$\begin{bmatrix} H & N \\ M & L \end{bmatrix}\begin{bmatrix} \Delta\delta \\ \Delta V \end{bmatrix} = \begin{bmatrix} \Delta P_g \\ \Delta Q_g \end{bmatrix} \quad (1)$$

Incremental power flow solution at all the solution point can be obtained by using "(1),". Where the using load flow Jacobian (J) is

$$J = \begin{bmatrix} H & N \\ M & L \end{bmatrix} \quad (2)$$

Voltage collapse condition is given as following [1]

$\lambda_{min}[H] \neq 0$

$\lambda_{min}[J] \neq 0$

where, $\lambda_{min}$ denotes minimum eigenvalue of [H] or [J] . Modal solution of incremental power flow "(1)," for $i^{th}$ eigenvalue can be written as follows:

$$\Delta V_{m,i} = \xi_i / \lambda_i \quad (3)$$

$\lambda_i$ - is the $i^{th}$ eigenvalue of load flow Jacobian.

$\xi_i$ - is the right eigenvector corresponding to $\lambda_i$.

$\Delta V_{m,i}$ - is the change in load bus voltage vector due to modal variation.

$$\Delta V_{m,i} = \sum_i (\alpha_i * \xi_i)/\lambda_i \quad (4)$$

$\alpha_i$ is a participation coefficient. If $\alpha_i = 0$, corresponding to minimum eigenvalue then such reactive load variation is not important. Since such variation of reactive power load does not contribute or aggregate the minimum eigenvalue, certain eigenvalue which are positive but have large magnitude even if these participation coefficient $\alpha_i$ are large, such variation of disturbance will not affect much to voltage instability. Hence, it is said that voltage collapse is modal that is voltage collapse occurs due to modal variation/ participation corresponding to minimum eigenvalue of load flow Jacobian.

It is obvious from "(4)," that all the eigenvalue of load flow Jacobian must be positive for the system to be voltage stable. When minimum eigenvalue is close to zero, a very small load change can results in very large voltage change. The operating point is considered as voltage unstable from load flow point of view. Hence, the magnitude of minimum eigenvalue must be as large as to have adequate stability margin.

Minimum eigenvalue of load flow Jacobian is obtained using following steps:

Step-1: Perform AC power flow using continuation power flow method and obtain load flow Jacobian [J].

Step-2: Obtain inverse load flow Jacobian.
$[JI] = [J]^{-1}$

Step-3: Select $E_0 = [1,0,0,0, \ldots\ldots\ldots\ldots,0]^T$

Step-4: Set iteration count $k = 1$.

## III. PROBLEM FORMULATION

The fitness function (F) to be selected is the maximization of minimum eigenvalue of load flow Jacobian with respect to these MW-generations.

$$F = \text{Max}\,\lambda_{min}(P_{g1}, P_{g2}, \ldots\ldots, P_{gn}, \ldots..P_{g\,NG}) \quad (5)$$

$(n \neq \text{slack bus})$

NG - Number of generator buses.

Above fitness function is optimized subject to following equality and inequality constraints.

(i) Power flow equations equality constraint:

$$P_{gi} - P_{di} - V_i \sum_{j=1}^{NB} V_j \left[G_{ij}\cos(\delta_i - \delta_j) + B_{ij}\sin(\delta_i - \delta_j)\right] = 0$$

$$Q_{gi} - Q_{di} - V_i \sum_{j=1}^{NB} V_j \left[G_{ij}\sin(\delta_i - \delta_j) - B_{ij}\cos(\delta_i - \delta_j)\right] = 0$$

$$i = 1,2,3,\ldots\ldots,NB \quad (6)$$

NB - Number of buses.

$P_{gi}, Q_{gi}$ - Real & reactive power generation at $i^{th}$ buses.

$P_{di}, Q_{di}$ - Real & reactive power demand at $i^{th}$ buses.

$V_i, V_j$ - Voltage magnitude of $i^{th}$ & $j^{th}$ buses.

$\delta_i, \delta_j$ – Phase angle of voltages of the $i^{th}$ & $j^{th}$ buses.

$G_{ij}, B_{ij}$ - Transfer conductance & susceptance between $i^{th}$ & $j^{th}$ buses.

(ii) Reactive power generation inequality constraint:

$$Q_{gi}^{min} \leq Q_{gi} \leq Q_{gi}^{max} \quad (7)$$

$$i = 1,2,3,\ldots\ldots,NG$$

$Q_{gi}^{min}$ and $Q_{gi}^{max}$ - Minimum & maximum limits of reactive power generator at $i^{th}$ buses.

(iii) Active power generation inequality constraint:

$$P_{gi}^{min} \leq P_{gi} \leq P_{gi}^{max} \quad (8)$$

$P_{g2}$ = 2.9981pu. Fitness function with best initial solution is obtained; F = 0.3995. Maximum numbers of generation is taken as 500 and terminated after 349 generations that are no improvement in fitness function. Table1 shows the comparison of active and reactive power generation under base case, best initial solution, BH, TLBO and PSO techniques. Table 2 shows the comparison of most sensitive load bus voltage magnitude with and without optimization using BH, TLBO and PSO techniques [18, 19]. Table 3 shows the comparison of BH with TLBO and PSO techniques based on fitness function, static stability limit and voltage stability margin. Table 5 shows the comparison of BH with TLBO and PSO techniques based on mean value, standard deviation, best value, worst value, frequency of convergence, standard error, length of confidence interval and confidence interval of fitness function [20]. "Fig. 1," shows a plot for comparison of the convergence of fitness function with respect to number of generation for BH, TLBO and PSO techniques.

Static voltage stability limit of the system is obtained using BH, TLBO and PSO techniques are 10.1834pu, 10.1634pu and 9.7975pu respectively. Voltage stability margin obtained at the end of optimization processes namely; BH, TLBO and PSO techniques are 23.9661%, 23.8169% and 20.9719% respectively. It is observed that BH gives much better global optimal results than TLBO and PSO techniques.

## VI. Conclusion

This paper has presented a new algorithm for improvement in voltage security margin under stressed condition by rescheduling of MW-generation subjected to operating and stability inequality constraints. This has been achieved using modal analysis and eigen sensitivities. Optimum MW-generation rescheduling has been obtained using developed BH algorithm. MW-generation rescheduling using the algorithm increases considerable voltage security margin and load bus voltages as well. Performance of the developed algorithm has been compared based on statistical inference with, TLBO and PSO techniques on standard IEEE 14-bus test system.

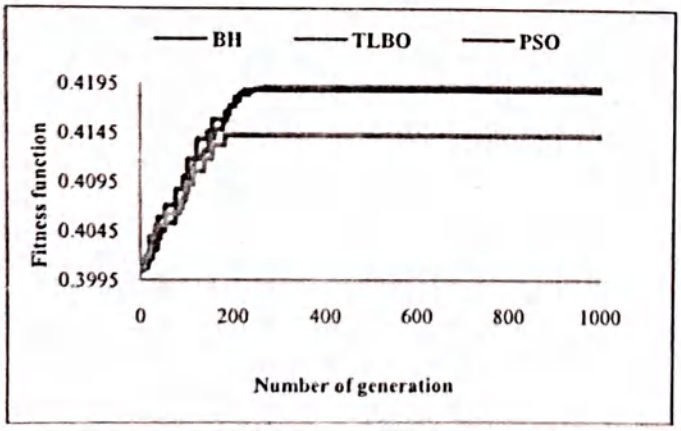


Fig. 1. Plot of convergence of fitness function with respect to number of generation using BH, TLBO & PSO algorithms for 14-bus system.

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**Table - 1. Active and reactive power generation under base case, best initial solution, BH, TLBO and PSO techniques for IEEE 14-bus test system ($S_{dt}$) = 7.7428pu .**

| Methodology | Active Power Generation | | Reactive Power Generation | |
|---|---|---|---|---|
| | Pg2 | Pg3 | Qg2 | Qg3 |
| Base Case | 1.7240 | 1.8370 | 1.9782 | 2.0550 |
| Best initial solution | 2.3709 | 2.9981 | 0.7855 | 0.9896 |
| BH | 2.5182 | 3.6984 | 0.9449 | 0.7536 |
| TLBO | 2.6085 | 3.9195 | 0.9524 | 0.7656 |
| PSO | 2.9219 | 3.0807 | 0.8621 | 0.8707 |

**Table - 2. Most sensitive load bus voltage magnitude using BH, TLBO and PSO algorithms for IEEE14-bus system ($S_{dt}$) = 7.7428pu.**

| Sr. No. | Most Sensitive Load Bus | Base Case | BH | TLBO | PSO |
|---|---|---|---|---|---|
| 1 | $V_5$ | 0.8153 | 0.9140 | 0.9143 | 0.9131 |
| 2 | $V_7$ | 0.8153 | 0.9140 | 0.9143 | 0.9131 |
| 3 | $V_9$ | 0.8185 | 0.9215 | 0.9217 | 0.9195 |
| 4 | $V_{10}$ | 0.8280 | 0.9334 | 0.9336 | 0.9306 |
| 5 | $V_{11}$ | 0.8511 | 0.9612 | 0.9615 | 0.9565 |
| 6 | $V_{10}$ | 0.8624 | 0.9761 | 0.9763 | 0.9701 |
| 7 | $V_{14}$ | 0.8183 | 0.9284 | 0.9287 | 0.9246 |

**Table - 3. Comparison of BH with SFLA and PSO techniques based on fitness function, static stability limit and voltage stability margin for IEEE 14-bus test system.**

| Sr. No. | Methodology | Fitness Function (pu) | Stability Limit (pu) | Voltage stability margin (%) |
|---|---|---|---|---|
| 1 | Base Case | 0.2593 | 8.3514 | 07.4645 |
| 2 | BH | 0.4191 | 10.1834 | 23.9661 |
| 3 | TLBO | 0.4188 | 10.1634 | 23.8169 |
| 4 | PSO | 0.4142 | 09.7975 | 20.9719 |

**Table -4. Comparison of BH with TLBO and PSO techniques based on statistical interence for IEEE14-bus system.**

| Optimization methods | Arithmetic mean value of the objective function | Standard deviation of objective function | Best value of objective function | Worst value of objective function | Frequency of convergence | Confidence level | Determined value for the Engg. Application | Standard error of the mean objective function | Confidence interval of the objective function | Length of confidence interval of the objective function |
|---|---|---|---|---|---|---|---|---|---|---|
| | $(\bar{F})$ | $(\sigma)$ | $(F_{best})$ | $(F_{worst})$ | | $(\gamma)$ | $(c)$ | $(\varepsilon)$ | $(\mu)$ | $(L)$ |
| **BH** | 0.4176 | 0.0013 | 0.4191 | 0.4144 | 12 | **0.95** | **2.0452** | 0.00061 | **0.4170≤ $\mu$ ≤ 0.4182** | 0.0025 |
| **TLBO** | 0.4172 | 0.0014 | 0.4188 | 0.4137 | 11 | 0.95 | 2.0452 | 0.00065 | 0.4165≤ $\mu$ ≤ 0.4178 | 0.0026 |
| **PSO** | 0.4097 | 0.0032 | 0.4142 | 0.4052 | 08 | 0.95 | 2.0452 | 0.00145 | 0.4082≤ $\mu$ ≤ 0.4111 | 0.0059 |

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# Evaluation of state probabilities based on deterministic criterion for a distribution system with distributed generation employing boot strapping technique

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*Abstract*— **This paper describes an algorithm for evaluating state probabilities of a distribution system having distributed generation. The probabilities have been classified as healthy, marginal and risky state probabilities based on a deterministic criterion which is based on load and capacity available. Uncertainties in the capacity available from substation and that from distributed generation have been considered. Uncertainties have been considered in two stages i.e. one stage considered the operable availability and the second stage considers random variations around the rated capacity available that of substation or distributed generation. To evaluate the state probabilities boot strapping has been incorporated in Monte Carlo simulation so as to enhance computational efficiency. The algorithm has been implemented on a sample distribution system incorporating annual load duration curve.**

***Keywords*— *Distribution system, distributed generations, Monte Carlo simulation, boot strapping, probability.***

*Abbreviations and Acronyms*

$\lambda_S$ Transition rate from up to down state of distribution substation.

$\mu_S$ Transition rate from down state to up state of distribution substation.

$A_S$ and $\overline{A}_S$ Availability and unavailability of distribution substation

$C_S$ Normally distributed Distribution substation capacity

$\overline{C}_S$ Distribution substation mean capacity

$\sigma_S$ Distribution substation standard deviation

$\lambda_i$ Transition rate from up to down state of $i^{th}$ DG unit

$\mu_i$ Transition rate from down state to up state of $i^{th}$ DG unit

$A_i$ and $\overline{A}_i$ Availability and unavailability of $i^{th}$ DG unit

$\overline{C}_{dg,i}$ and $\sigma_i$ Mean capacity and standard deviation of $i^{th}$ DG unit

NDG Number of DG units are present in distribution system

$C_d$ Total capacity available from DG units

$C_T$ Total capacity sample of the distribution system with DG

$L_m$ $m^{th}$ step load level

$D_m$ $m^{th}$ time duration (p.u.) for occurrence of particular $m^{th}$ step load level

$N_H$, $N_M$ and $N_R$ Number of samples observed in healthy, marginal and risky state.

NS Total number of samples

$\hat{p}_H$, $\hat{p}_M$ and $\hat{p}_R$ Probability in healthy, marginal and risky state respectively.

$\beta$ Coefficient of variation

'NB' Bootstrap data set for the system states by re-sampling from data set

## I. INTRODUCTION

Distribution systems were designed to deliver electric energy to the consumer without any generation on these systems, so adequate performance of the distribution system depends on substation capacity/power available. Due to many uncertainties present such as transmission line outages, generator maintenance etc., the power available from transmission network via distribution substation to the distribution network is a random variable. It is natural choice from central limit theorem to assume the capacity available from substation as normally distributed. Due to deregulation in electric markets, generating units of small size ranging from few KW to few MW synchronized at 11 KV bus in distribution system, usually owned and controlled by customers known as distributed generation (DG). A consequence of these developments is that there is an increasing amount of energy generated at local distribution level by independent non-utility generators and increasing mber of new type of energy source, particularly renewable and CHP (combined heat power) schemes being developed. In this sense even distribution system have become composite system [1,2]. The technical merits [3] associated with the implementation of distributed generation includes in maintaining voltage profile, release of system capacity, energy loss reduction and improvement of composite system reliability.

Researchers have attempted the issue of generating capacity reliability evaluation but very limited efforts have been made [4,5,6] in adequacy assessment of distribution system accounting distributed generations DG. Hegazy et. al. [4] probably were the first to assess the adequacy of composite distribution system. Arya and Koshti [5] used safety index for planning distributed generation in a distribution system. Costa et. al. [6] identified the situations where the existence of a micro grid may reduce the interruption rate and duration and thus improve the reliability indices of the distribution network. In view of adequacy assessment there is growing interest in combining deterministic considerations with probabilistic essment in order to evaluate the "system well-being" [7] of a composite generation and transmission system and to evaluate the likelihood not only of entering a complete failure state but also the likelihood of being very close to trouble. Wangdee et. al. [7] presented bulk electric system well-being analysis using sequential Monte Carlo simulation. Amaral et. al. [8] developed efficient method for composite power system well-being evaluation based on non-sequential monte carlo simulation. Ahmadi-Khatir et. al. [9] has developed a methodology for allocation of cost associated with providing spinning reserve among distribution companies based on their demands and desired reliability using well being analysis. Arya et.al. [10] used a non-sequential Monte carlo simulation technique for frequency-duration calculation of a composite distribution system.

Various re-sampling technique are available in literature related to statistics. If these techniques are employed along with MCS then considerable reduction in computational time can be achieved. Boot strapping is such a re-sampling methodology [11, 12, 13] which can be utilized for efficient evaluation of state probabilities.

In view of above, it is inferred that well being analysis framework for adequacy assessment of distribution system having DG has not been addressed. So this paper addresses the well being analysis framework also known as 'health analysis' for distribution system adequacy assessment involving DG. A deterministic criteria has been incorporated with probabilistic approaches in evaluation of reliability indices for three states healthy state, marginal state and risky state that are estimates of the risk associated with the system states using well being analysis framework. The boot strapping and Monte Carlo Simulation (MCS) techniques have been used to evaluate state probabilities. Discrete availability and then uncertainty in capacity availabilities continuous distribution have been considered.

## II. DISTRIBUTION SUBSTATION CAPACITY MODELING

Due to many uncertainties present, the power available from transmission network via distribution substation to the distribution network is a random variable. So the substation will results in a random contribution to the system capacity. A two state model (up and down) [1] is used to model the operation of distribution substation. Transition rate from up to down state and down state to up state are assumed constants. Based on these transition rates availability and unavailability of substation may be calculated as:

$$A_S = \frac{\mu_S}{\lambda_S + \mu_S} \quad (1)$$

$$\overline{A}_S = \frac{\lambda_S}{\lambda_S + \mu_S} \quad (2)$$

$\lambda_S$ is the transition rate from up to down state of distribution substation.

$\mu_S$ is the transition rate from down state to up state of distribution substation.

$A_S$ and $\overline{A}_S$ are availability and unavailability of distribution substation.

Now if distribution substation is available then capacity $C_S$ is assumed to be a normally distributed with mean $\overline{C}_S$ and standard deviation $\sigma_S$ and expressed as:

$$f(C_S) = \frac{1}{\sqrt{2\pi}\sigma_S} e^{-0.5\left(\frac{C_S - \overline{C}_S}{\sigma_S}\right)^2} \quad (3)$$

Based on the availability of substation, capacity samples are obtained from distribution function explained in above section.

## III. DISTRIBUTED GENERATION MODELING

Different customers would have different strategies for operating their DG unit and accordingly the process of turning on and off each DG unit will be random process. This process will results in a random contribution to the overall system capacity. Markov modeling is adopted to calculate the availability of each DG unit as follows:

$$A_i = \frac{\mu_i}{\lambda_i + \mu_i} \tag{4}$$

$$\overline{A}_i = \frac{\lambda_i}{\lambda_i + \mu_i} \tag{5}$$

$\lambda_i$ is the transition rate from up to down state of $i^{th}$ DG unit.

$\mu_i$ is the transition rate from down state to up state of $i^{th}$ DG unit.

$A_i$ and $\overline{A}_i$ are availability and unavailability of $i^{th}$ DG unit.

It is assumed that, NDG number of DG units are present in distribution system. Capacity available from each DG unit is assumed as continuous random variable with normal distribution function. For $i^{th}$ DG unit distribution function can be expressed as follows:

$$f(C_{dg,i}) = \frac{1}{\sqrt{2\pi}\sigma_i} e^{-0.5\left(\frac{C_{dg,i} - \overline{C}_{dg,i}}{\sigma_i}\right)^2} \tag{6}$$

Where $\overline{C}_{dg,i}$ and $\sigma_i$ represents mean capacity and standard deviation of $i^{th}$ DG unit.

Based on the availability of DG units, total capacity available from DG units can be obtained as:

$$C_d = \sum_k C_{dg,k} \quad k \in \text{set of available units} \tag{7}$$

Where $C_{dg,k}$ is capacity sample of $k^{th}$ DG unit as obtained from (6).

## IV. MODELING OF DISTRIBUTION SYSTEM CAPACITY

The total available capacity of a distribution system is random variable and is given as:

$$C_T = C_S + C_d \tag{8}$$

Where

$C_T$ is the total capacity sample of the distribution system with DG.

$C_S$ is the capacity sample available due to distribution substation alone.

$C_d$ is the capacity sample available from the DG.

## V. LOAD MODELING

The load duration curve (LDC) is not normally used directly in a composite system adequacy evaluation due to the extremely large required computation time. Instead, the LDC is usually approximated represented by load model which possess fewer steps. A typical seven step load duration curve is shown in Fig-1.

Where $L_m$ represents the $m^{th}$ step load level and $D_m$ time duration (in p.u.) of the respective step load level. The time duration may also have stochastic nature for which a particular step load is available. So a uniformly distributed random number u[0,1] can be generated to represent time duration for existence of particular step load level.

## VI. SYSTEM STATE MODELING BASED ON DETERMINISTIC CRITERION

Based on a deterministic criterion the system state is characterized as healthy state, marginal state or risky state [7,10] as shown in Fig-2.

In our present paper following criterion defines the three states which depends on capacity to load ratio at a particular time point.

i. Healthy state if
$$C_T / L_m \geq 1.15 \tag{9}$$

ii. Marginal state if
$$1 \leq C_T / L_m < 1.15 \tag{10}$$

iii. Risky state or state of load exceeding the capacity if
$$C_T / L_m < 1 \tag{11}$$

Hence the deterministic criterion classify a particular operating state. In view of this capacity is 1.15 times of load or more then that state is considered as healthy and where the ratio is less than unity the state is considered as risky and between two the state is considered as marginal.

The computational algorithm for reliability evaluation using MCS is given as follows.

## VII. COMPUTATIONAL ALGORITHM USING MCS

The MCS is used for reliability evaluation of distribution system in healthy, marginal and risky state as explained in following steps:

**Step-1:** Evaluate availability and unavailability of distribution substation using relation (1) and (2).

**Step-2:** Generate a uniformly distributed number $U_1$ in the range $[0,1]$ for deciding status of distribution substation.

If $0 < U_1 \leq A_S$ then distribution substation (DS) capacity is available, otherwise the DS is not available and capacity available is zero.

**Step-3:** Evaluate capacity sample of distribution substation using Normal distribution function using relation (3).

**Step-4:** Evaluate availability and unavailability of DG units using relation (4) and (5).

**Step-5:** Generate 'NDG' numbers from uniform distribution in the range [0, 1]. If for a unit $0<U_i \le A_i$, the unit and capacity is available, otherwise the unit is not available and capacity available from the DG is zero.

**Step-6:** Identify the DG units available as in previous steps, generate DG capacity samples using relation (6) for available DG units and calculate DG capacity available using relation (7).

**Step-7:** Obtain sample of total capacity of distribution system $C_{T_i}$ relation (8).

**Step-8:** Generate a uniformly distributed random digit $U_m[0, 1]$ showing $m^{th}$ time duration $D_m$(p.u.) for occurrence of particular $m^{th}$ step load level as follows:

If, $T_m \le U_m \le T_{m+1}$ then step load level $L = L_m$, for m=1,…,NL

Where NL represents total number of steps of load level.

$T_m$ and $T_{m+1}$ represents starting and end time of $m^{th}$ step load level respectively in percent.

**Step-9:** Define sample states using deterministic criteria explained in (9), (10) and (11).

Repeat steps 1-9 for large number of times say (NS=10,000).

**Step-10:** Evaluate probabilities of three states given below:

$$\hat{p}_H \cong \frac{N_H}{NS} \tag{12}$$

$$\hat{p}_M \cong \frac{N_M}{NS} \tag{13}$$

$$\hat{p}_R \cong \frac{N_R}{NS} \tag{14}$$

Where $N_H$, $N_M$ and $N_R$ denotes number of samples observed in healthy, marginal and risky state. NS denotes total number of samples generated. $\hat{p}_H$, $\hat{p}_M$ and $\hat{p}_R$ represents the probability in healthy, marginal and risky state respectively.

**Step-11:** Check for convergence using coefficient of variation ($\beta$) which is calculated for each state probabilities as follows

$$\beta = \sqrt{\frac{(1-\hat{p})}{\hat{p}\cdot NS}}$$

$\hat{p}$ is state probabilities i.e. $\hat{p}_H$ or $\hat{p}_M$ or $\hat{p}_R$. In all three states the coefficient of variation should be less than threshold values say 0.04, otherwise repeat the procedure from step-1.

## VIII. Boot Strapping: An Application for Evaluating Healthy, Marginal and Risky State Probabilities

Many researchers have used boot strapping for enhanced sampling in MCS [11, 12, 13, 14, 15] to reduce execution time. Boot strapping is re-sampling technique with replacement in which a given data set is used to re-sample randomly to provide another data set. This is repeated for number of times (NB). The algorithm is explained in following steps-

a) Obtain a data set of states using MCS as explained in previous section for a less number of time say NS=500 as explained in step-9 and 10 in sec.-VII. The data set will be represented as-

$$SS:\{0,\ 0,\ 1,\ 2,\ 0,\ldots\ldots\} \Rightarrow \{n_i\} \tag{15}$$

$n_i = 0$, if the state is healthy

$n_i = 1$, if the state is marginal

$n_i = 2$, if the state is risky as defined in previous portion.

b) Obtain 'NB' bootstrap data set for the system states by re-sampling from data set given in step-(a) by replacements. It will be represented as-

$$SB_I:\{n_i\} \qquad i=1,\ldots..,NS \tag{16}$$
$$I=1,\ldots.,NB$$

c) Calculate for each boot strap sample state probabilities as follows-

$$\hat{p}_{H,I} \cong \frac{1}{NS}\sum F_i \tag{17}$$

$F_i = 1$ if $n_i = 0$

$F_i = 0$ otherwise

$$\hat{p}_{M,I} \cong \frac{1}{NS}\sum F_i \tag{18}$$

$F_i = 1$ if $n_i = 1$

$F_i = 0$ otherwise

$$\hat{p}_{R,I} \cong \frac{1}{NS}\sum F_i \tag{19}$$

$F_i = 1$ if $n_i = 2$

$F_i = 0$ otherwise

In above $F_i$ is termed as test function.

d) Calculate mean value of state probabilities as follows

$$\hat{p}_H \cong \frac{1}{NB}\sum_{I=1}^{NB}\hat{p}_{H,I} \tag{20}$$

$$\hat{p}_M \cong \frac{1}{NB}\sum_{I=1}^{NB}\hat{p}_{M,I} \tag{21}$$

$$\hat{p}_R \cong \frac{1}{NB}\sum_{I=1}^{NB}\hat{p}_{R,I} \tag{22}$$

The sampling distribution of $\hat{p}_{H,I}$, $\hat{p}_{M,I}$ and $\hat{p}_{R,I}$ approaches to normal distribution due to applicability of central limit theorem [ 16]. Convergence is ascertained using coefficient of variations for each state probabilities. The implementation of the boot strapping algorithm for obtaining state probabilities is given in results section.

## IX. Results and Discussions

A IEEE-Reliability test system (RTS) [17] is considered as sample distribution system with substation availability as 0.95.

aper Id:

apacity has been assumed as normally distributed for ubstation of given mean and standard deviation given as ollows:

substation of given mean and standard deviation given as follows:

$\overline{C}_S = 2800$ MW, $\sigma_S = 150$ MW

Capacity samples for distribution substation has been obtained as follows:

$$C_s = 150 \cdot N(0,1) + 2800.0 \qquad (23)$$

Where N(0,1) is standard normally distributed random number having mean zero and standard deviation one.

To represent the status of distribution substation a uniformly distributed random digit

$U_1[0,1]$ is used and status is decided based on the following criteria:

$0<U_1 \le 0.95$ Distribution substation is in up state, otherwise in down state.

Five DG units have been considered in this paper, capacity of DG units are assumed normally distributed with respective ...ilabilities are shown in Table I[4]. Capacity sample for DG units are obtained as follows:

$$C_{dg,i} = 8 \cdot N(0,1) + 80.0, \qquad i = 1,\ldots, 5$$

Uniformly distributed random digits, $U_2$ to $U_6$ are generated to decide the Status of five DG units given as follows:

$0<U_{i+1} \le A_i$ $i^{th}$ DG is in up state, otherwise in down state for i=1,...,5

A simplified seven step load model based on the IEEE-RTS annual load duration curve [16] is considered for well being analysis of distribution system having an annual peak of 2850 MW (1 p.u.) is shown in Table II.The graphical plot of seven step annual load duration curve is considered as shown in Fig-1 with $l_{min}$=0.3 p.u. and $l_{max}$=1.0 p.u.. Time is represented in p.u. for annual load duration curve of seven step load model.

Another uniformly distributed random number $U_7$ can be generated to represent the time duration (p.u.) for existence of particular step load level explained as follows:

$0.00000 \le U_7 < 0.01312$ Step load level $L_0$ is available

$...1312 \le U_7 < 0.12392$ Step load level $L_1$ is available

$0.12392 \le U_7 < 0.28892$ Step load level $L_2$ is available

$0.28892 \le U_7 < 0.52032$ Step load level $L_3$ is available

$0.52032 \le U_7 < 0.73532$ Step load level $L_4$ is available

$0.73532 \le U_7 < 0.96102$ Step load level $L_5$ is available

$0.96102 \le U_7 < 1.00000$ Step load level $L_6$ is available

The three state probabilities have been estimated using MCS as well as boot strapping technique and results are shown in Table III. The state probabilities have been obtained without DG and DG in the distribution system. Number of samples required for convergence using MCS are 10000. Number of samples used in boot strapping is 500. Number of re-sampling samples (NB) are 200 for convergence. CPU time required is given in Table IV. It is observed that CPU time required using boot strapping is 46% of that required using MCS.

It is observed there is significant improvement of probability in healthy state and reduction in probability of risky state with addition of DG capacity in distribution system.

## X. CONCLUSIONS

A methodology has been developed for adequacy assessment of a distribution system having DG sources. A deterministic criterion have been used to classify the three state probabilities i.e. healthy, marginal and risky. Two step uncertainties have been considered in capacity modeling of distribution substation. The first uncertainty is by any random outage of distribution substation. This is mainly a binary state with certain probability of availability. The second stage considers continuous uncertain variation around mean value of capacity. This has been represented as normal distribution. Similar two stage modeling has been adopted for each DG sources. The three state probabilities have been obtained using MCS and using boot strapping technique. Computationally boot strapping technique have been found quite efficient and results obtained are in close agreement with those obtained using MCS. Significant improvement is observed in adequacy of distribution system with DG.

TABLE I. AVAILABILITY AND CAPACITY OF DG

| Unit No. *(i)* | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Failure Rate $\lambda_i$ (failures/ hr) | 0.001 | 0.0024 | 0.003 | 0.004 | 0.007 |
| Repair Rate $\mu_i$ (repairs/hr) | 0.003 | 0.005 | 0.006 | 0.004 | 0.005 |
| Availability $A_i$ | 0.750 | 0.676 | 0.666 | 0.500 | 0.417 |
| Mean capacity (MW) $\overline{C}_{dg,i}$ | 80.00 | 80.00 | 80.00 | 80.00 | 80.00 |
| Standard deviation (MW) $\sigma_i$ | 8.000 | 8.000 | 8.000 | 8.000 | 8.000 |

TABLE II. SEVEN STEP LOAD MODEL BASED ON IEEE-RTS ANNUAL LOAD DURATION CURVE

| Sr. No. | Time (p.u.) | | Step load level (MW) | |
|---|---|---|---|---|
| | From | To | | |
| 1 | 0 | 0.01312 | $L_0$ | 2850.0 |
| 2 | 0.01312 | 0.12392 | $L_1$ | 2793.0 |
| 3 | 0.12392 | 0.28892 | $L_2$ | 2736.0 |
| 4 | 0.28892 | 0.52032 | $L_3$ | 2679.0 |
| 5 | 0.52032 | 0.73532 | $L_4$ | 2622.0 |
| 6 | 0.73532 | 0.96102 | $L_5$ | 2565.0 |
| 7 | 0.96102 | 1.00000 | $L_6$ | 2508.0 |

TABLE III. STATE PROBABILITIES USING BOOT STRAPPING AND MCS TECHNIQUE

| State | $\hat{p}_H$ using MCS | $\hat{p}_M$ using MCS | $\hat{p}_R$ using MCS | $\hat{p}_H$ using boot strapping | $\hat{p}_M$ using boot strapping | $\hat{p}_R$ using boot strapping |
|---|---|---|---|---|---|---|
| Without DG | 0.0691 | 0.6858 | 0.2451 | 0.056 | 0.712 | 0.2320 |
| With DG | 0.4403 | 0.4859 | 0.0738 | 0.458 | 0.4680 | 0.0740 |

TABLE IV. CPU TIME AND NUMBER OF SAMPLES REQUIRED USING MCS AND BOOT STRAPPING TECHNIQUE

| State | Using MCS | Using boot strapping |
|---|---|---|
| CPU time required (seconds) | 1.79 | 0.82 |
| Number of samples | 10000 | 200 |

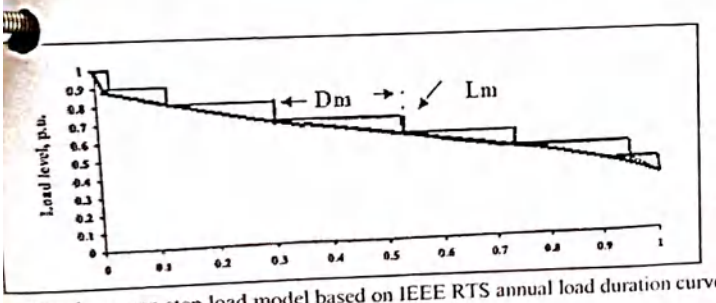


Fig. 1. seven step load model based on IEEE RTS annual load duration curve

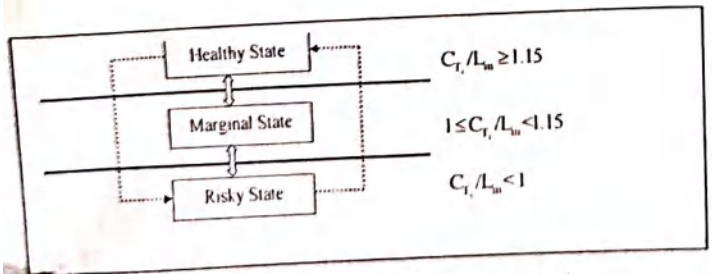


Fig. 2. Classification of state based on deterministic criteria

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