DECLARATION

I declare that the thesis entitled “Synthesis and characterization of phototrophic microbial granules for bioremediation” submitted by me for the degree of Doctor of Philosophy (Ph.D.) is the record of work carried out by me during the period from December, 2003 to December, 2011 under the guidance of Dr V. P. Venugopalan and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other University or other similar institution of Higher Learning.

Signature of the Candidate

( RAJESH KUMAR )
Contents

Contents in Detail........................................................................................................... ii
Chapter 1 : Introduction and Thesis Outline.................................................1
Chapter 2 : Microbial Granulation in Shake Flask Reactors .... 26
Chapter 3 : Optimizing and Improving Microbial Granulation 53
Chapter 4 : Development of Self-sustaining Phototrophic Granular Biofilms ........................................................................................................ 75
Chapter 5 : Degradation of Phenol by Phototrophic Granules ..111
Chapter 6 : Synthesis and conclusions.......................................................... 137
Summary ........................................................................................................ 149
References ............................................................................................................... 151
## Contents in Detail

**Chapter 1 : Introduction and Thesis Outline** ............................................. 1

1.1 Introduction............................................................................................................ 1

1.1.1 Biofilms and their role in bioremediation......................................................... 1

1.1.2 Self-aggregated substratum less biofilms......................................................... 3

1.1.3 Activated sludge process: use in bioremediation and limitations....................... 4

1.1.4 Microbial granulation......................................................................................... 5

1.1.5 Microbial aerobic granulation in different reactor systems............................. 6

1.1.6 Sequencing batch reactors ................................................................................ 7

1.1.7 Experimental studies on aerobic granulation..................................................... 8

1.1.8 Bioremediation with the help of aerobic granules ............................................ 12

1.1.9 Summary of reviews on aerobic granulations and knowledge gaps ......... 15

1.1.10 Phototrophic biofilms....................................................................................... 16

1.1.11 Photobioreactor systems .................................................................................. 18

1.1.12 Phototrophic biofilms in bioremediation ......................................................... 19

1.1.13 Knowledge gaps and limitations of aerobic granulation technology and phototrophic biofilms.............................................................................................................. 21

1.2 Objectives .............................................................................................................. 23

1.3 Organization of the thesis ...................................................................................... 25

**Chapter 2 : Microbial Granulation in Shake Flask Reactors ....** 26

2.1 Abstract .................................................................................................................. 26

2.2 Introduction.............................................................................................................. 26

2.3 Objectives .............................................................................................................. 28

2.4 Materials and methods .......................................................................................... 29

2.4.1 Preliminary experiments..................................................................................... 29

2.4.2 Culture conditions and reactor operation......................................................... 29

2.4.3 Wastewater......................................................................................................... 30

2.4.4 Samples and analysis.......................................................................................... 30

2.4.5 Microbiological diversity of MAGs.................................................................... 31

2.5 Results .................................................................................................................... 32

2.5.1 Aerobic condition.............................................................................................. 32

2.5.2 COD reduction in wastewater............................................................................ 34

2.5.3 Nutrient release in effluents............................................................................... 35
Chapter 2: MAGs formation and characterisation

2.5.4 Sludge volume index (SVI) ................................................................. 36
2.5.5 Granule formation and characterisation ................................................ 37
2.5.1 MAGs formation in BWW ................................................................. 38
2.5.2 MAGs formation in MWW ................................................................. 39
2.5.3 MAGs formation in SWW ................................................................. 43
2.5.4 Microbial diversity of granule ............................................................ 45
2.6 Discussion .......................................................................................... 46
2.6.1 Dissolved oxygen ............................................................................. 46
2.6.2 COD removal .................................................................................... 48
2.6.3 Nutrient removal .............................................................................. 48
2.6.4 SVI and settling velocity of granules .................................................. 49
2.6.5 Morphology of granules .................................................................. 50
2.6.6 Microbial diversity of MAGs .............................................................. 51
2.7 Conclusions ......................................................................................... 51

Chapter 3: Optimizing and Improving Microbial Granulation 53

3.1 Abstract ............................................................................................... 53
3.2 Introduction ........................................................................................... 54
3.3 Objectives ............................................................................................. 55
3.4 Material and methods .......................................................................... 56
3.4.1 Reactor setup for optimisation of shear force ..................................... 56
3.4.2 Reactor setup and inoculum for formation of MAGs from biofilm biomass 57
3.4.3 Samples and analysis ....................................................................... 57
3.5 Results .................................................................................................. 58
3.5.1 Optimization of shear force .............................................................. 58
3.5.2 Improving granulation in shake flask reactors by using biofilm-derived biomass and quorum sensing molecules ................................................. 63
3.6 Discussion ........................................................................................... 70
3.6.1 Optimization of shear force .............................................................. 70
3.6.2 Improving granulation by using novel biomass and quorum sensing chemicals ................................................................. 72
3.6.3 Microbial diversity of granules ......................................................... 73
3.7 Conclusion .......................................................................................... 73
Chapter 4: Development of Self-sustaining Phototrophic Granular Biofilms

4.1 Abstract ................................................................................................................... 75
4.2 Introduction ............................................................................................................. 76
4.3 Objectives ............................................................................................................... 77
4.4 Material and methods ........................................................................................... 78
  4.4.1 Preliminary experiments ............................................................................... 78
  4.4.2 Reactor setup .................................................................................................. 78
  4.4.3 Biomass and cultures conditions for studies in shake flask reactors .... 79
  4.4.4 Biomass and culture conditions for studies in bubbled column reactors 80
  4.4.5 Sampling and analysis .................................................................................. 82
  4.4.6 Summary of reactor operation ....................................................................... 82
4.5 Results ................................................................................................................... 85
  4.5.1 Preliminary experiments ............................................................................... 85
  4.5.2 Granulation of pure cultures in shake flask reactors ................................. 85
  4.5.3 SSPG formation from activated sludge and mixed algal consortia in shake flask reactors ........................................................................................................ 86
  4.5.4 SSPG development in bubbled column reactor ........................................... 90
  4.5.5 Storage stability of SSPGs ............................................................................. 103
4.6 Discussion ............................................................................................................. 104
  4.6.1 SSPGs development with pure cultures ....................................................... 105
  4.6.2 Formation of SSPGs in shake flask reactors ............................................... 106
  4.6.3 Development of SSPGs in bubbled column SBRs ....................................... 107
  4.6.4 Storage of SSPGs ......................................................................................... 110
4.7 Conclusion ............................................................................................................ 110

Chapter 5: Degradation of Phenol by Phototrophic Granules

5.1 Abstract ................................................................................................................. 111
5.2 Introduction ........................................................................................................... 111
5.3 Objectives ............................................................................................................. 113
5.4 Materials and methods ....................................................................................... 113
  5.4.1 Preliminary experiments ............................................................................. 113
  5.4.2 Bioaugmentation for phenol degradation in shake flask reactors ............. 113
5.4.3 Biodegradation of phenol in bubbled column SBRs ........................................ 114
5.4.4 Effect of light availability on phenol degradation ......................................... 115
5.4.5 Loss of function and recovery ...................................................................... 116
5.5 Results ........................................................................................................... 116
  5.5.1 Preliminary experiments ............................................................................ 116
  5.5.2 Bioaugmentation of SSPGs for phenol degradation .................................... 116
  5.5.3 Development of SSPGs for phenol degradation ......................................... 118
  5.5.4 Degradation of phenol by SSPGs .............................................................. 121
  5.5.5 Effect of phenol on SSPGs: ....................................................................... 125
  5.5.6 Role of availability of light in phenol degradation function of SSPGs .......... 128
  5.5.7 Recovery of phenol degradation function .................................................. 130
5.6 Discussion ..................................................................................................... 132
  5.6.1 Bioaugmentation of SSPGs for phenol degradation .................................... 132
  5.6.2 Development of SSPGs for phenol degradation ......................................... 132
  5.6.3 Phenol degradation by SSPGs ................................................................. 133
  5.6.4 Effect of phenol on SSPGs ....................................................................... 134
  5.6.5 Role of availability of light in phenol degradation function of SSPGs .......... 134
  5.6.6 Recovery of phenol degradation function of SSPGs .................................. 135
5.7 Conclusion .................................................................................................... 135

Chapter 6: Synthesis and conclusions ............................................................... 137

  Aerobic granulation .......................................................................................... 138
  Microbial granulation in shake flask SBRs ....................................................... 139
  Optimization of microbial granulation in shake flask SBRs ........................... 140
  Development of self-sustaining phototrophic granular biofilms .................... 142
  Degradation of phenol by photosynthetic granules ......................................... 143
  Conclusions ...................................................................................................... 145

  Proposal for future work .................................................................................. 146

Summary 149

References 151
List of Figures

Figure 1-1: Schematic of biofilm formation, maturation and dispersal .................... 2
Figure 1-2: Schematic of activated sludge process used in wastewater treatment ...... 4
Figure 1-3: A typical SBR cycle consisting of filling, reaction, settling, decanting and idling phases ................................................................. 7
Figure 1-4: The process of formation of aerobic granules .................................. 12
Figure 1-5: Natural Phototrophic biofilms ....................................................... 17
Figure 1-6: Schematic of phototrophic biofilm formation indicating energy and material flow ............................................................................ 17
Figure 1-7: Principle of photosynthetic oxygenation in BOD removal processes .... 20
Figure 1-8: Potential benefits of using phototrophs in bioremediation of wastewater 21
Figure 2-1: Granular biomass in SFSBRs with A) MWW and B) SWW .................. 32
Figure 2-2: Changes in dissolved oxygen levels in the shake flask reactors within a single cycle time ................................................................. 33
Figure 2-3: Percentage reduction in COD in different wastewaters .................. 34
Figure 2-4: Concentration of ortho-phosphates in the effluents ......................... 35
Figure 2-5: Ammonium-N (NH4+-N) and Nitrate-N (NO3--N) in the effluents .... 36
Figure 2-6: Changes in sludge volume index (SVI) in all reactors during the study period ......................................................................................... 37
Figure 2-7: MAGs in bulk water and after 30s settling in an Imhoff cone A) BWW, B) MWW and C) SWW ............................................................... 38
Figure 2-8: Microbial aerobic granule formation in BWW .................................. 38
Figure 2-9: Shape descriptors of MAGs developed in BWW .......................... 40
Figure 2-10: Microbial aerobic granule formation in MWW ............................ 41
Figure 2-11: Shape descriptors of MAGs developed in MWW ......................... 42
Figure 2-12: Microbial aerobic granule formation in synthetic wastewater .... 43
Figure 2-13: Shape descriptors of MAGs developed in SWW ......................... 44
Figure 2-14: Genetic diversity of MAGs developed in different wastewaters ...... 45
Figure 3-1: Image sequence showing the granules developed in different reactors 58
Figure 3-2: Mean perimeter (A) and mean circularity (B) of granules ................ 60
Figure 3-3: Distribution of granules developed in different reactors by their size at the end of study ................................................................. 60
Figure 3-4: Average settling velocity of granules developed in different reactors at the end of study. Error bar present standard deviation of sample .............. 61
Figure 3-5: Settled sludge volume (SSV) and Sludge volume index (SVI) ........... 62
Figure 3-6: Percentage of COD removal by different reactors ........................ 62
Figure 3-7: Scanning electron microscope images of granules developed in 100rpm reactor ................................................................................. 63
Figure 5-1: Results of preliminary experiments showing degradation of phenol, p-nitrophenol and Congo red.................................................................117
Figure 5-2: Degradation of phenol by previously formed SSPGs..........................118
Figure 5-3: Image sequence showing formation of SSPGs in column reactors........119
Figure 5-4: Structural attributes (mean perimeter and mean circularity) of granules120
Figure 5-5: The physical parameters of bulk water (temperature, DO and pH) ........120
Figure 5-6: Distribution of granules by size (A) and circularity (B) .........................121
Figure 5-7: Changes in concentration of phenol in control reactors....................122
Figure 5-8: Graph sequence showing percentage of phenol remaining in the reactor as a function of cycle time in PDR2 reactor in different cycles........123
Figure 5-9: Graph sequence showing percentage of phenol remaining in the reactor as a function of cycle time in PDR3 reactor in different cycles........124
Figure 5-10: Graphs showing the concentration of phenol in inlet, in the reactors after 1 h and 3 h and outlet concentration (12h) in PDR2 (A) and PDR3 (B) reactors.................................................................125
Figure 5-11: MLSS (A) and SVI (B) of PDR1, PDR2 and PDR3 .........................126
Figure 5-12: Image sequence showing changes in sludge structure with time........126
Figure 5-13: Shape descriptors of the granules in PDR1, PDR2 and PDR3 reactors after starting phenol addition .................................................................128
Figure 5-14: Graph sequence describing degradation of phenol in 8 h cycles ........129
Figure 5-15: Degradation profile of phenol in PDR2 and PDR3 reactors..........130
Figure 5-16: Restoration of phenol degradation function of SSPGs. ................131
List of Tables

Table 2-1: Chemical oxygen demand of the waste waters used in the study .......... 30
Table 2-2: Comparison of different methods of aerobic microbial granule formation. 47
Table 2-3: Number of granules and surface area offered by granules in different wastewaters at steady state of reactor ................................. 51
Table 3-1: Shear force in different reactors ............................................................ 56
Table 4-1: Details of operational parameters of shake flask reactors ................ 83
Table 4-2: Details of operational parameters of bubbled column reactors .......... 84
Table 5-1: Detail of reactor operation to study effect of availability of light on functionality of SSPGs ......................................................... 116