

**PROCESS ASSESSMENT OF BIO-ENERGY OPTIONS
(HYDROGEN AND METHANE) USING MICROBES
WITH INDUSTRIAL WASTE WATER**

**SUMMARY
of
THESIS**

**SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW**

**BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY**



LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

**FOR THE DEGREE OF
Doctor of Philosophy
IN
ENVIRONMENTAL SCIENCE**

Submitted by

Virendra Kumar

Enrolment No: 239/11

UNDER SUPERVISION OF

Dr. Richa Kothari Tyagi

Assistant Professor

**DEPARTMENT OF ENVIRONMENTAL SCIENCE
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY, NAAC ACCREDITATION 'A' GRADE)
LUCKNOW-226 025**

2015

Executive Summary

Biological processing of industrial waste materials to produce bioenergy options as a product to fulfill the objective of “green technologies and sustainable development”, as such, is widely researched. Among various bioenergy options like biofuel, biogas, biohydrogen *etc.*, biological hydrogen and methane production generates a new arena of waste treatment with simultaneously bioenergy production. Present hydrogen production methods can be grouped into two broad categories: conventional and alternative. Conventional hydrogen production methods mainly involve steam reforming (methane, ethane, butane *etc.*), a process responsible for large amounts of CO₂ emissions whereas, alternative methods of hydrogen production includes electrolysis of water, biophotolysis and biological hydrogen production from organic materials. Among all the novel processes of hydrogen production, biological hydrogen production has two main advantages over the conventional methods; it generate less green house gases and couples the metabolic activity of hydrogen-producing micro-organisms with the use of organic rich human-derived wastes, such as domestic and industrial wastewaters as the substrates for bioenergy (hydrogen and methane) production. So, bioenergy production technologies are very efficient in terms of economical, environmental and sustainable point of view. The lacunas of these technologies are inefficient production rate, unavailability of efficient microbes and optimization of process parameters *etc.*

Anaerobic/dark fermentation and photosynthetic degradation are the two most widely studied biohydrogen production techniques. Among these processes, dark fermentation is more advantageous due to its early hydrogen evolution rate. From literature, we are able to know that inoculums, substrate, reactors type, nutrients concentration, temperature, and pH are the main influencing factors in biohydrogen production routes. The biggest challenge over the next few years in fermentative hydrogen production will be to reduce the cost optimization of substrate concentration (industrial organic waste materials- solid and liquid) and selection of bacterial inoculums (pure/mixed) to improve the biohydrogen yields in particular.

On seeing the scenario of biological hydrogen production, this study aims to analyze the effect of some process parameter with production mechanism and cost-optimization with the advancement in biohydrogen production researches and followed by the fruitful suggestions for the future work of fermentative hydrogen production. Hence, the main objective of this thesis is to assess the process of bioenergy options (hydrogen and methane) using industrial waste (solid and liquid) in

integration with selected pure bacterial strain in general and effect of process and operational parameters on growth, substrate utilization and gas production (hydrogen and methane) in particular with validation through kinetic studies.

The thesis has been divided into following seven chapters:

1. Introduction
2. Review of literature
3. Materials and Methods
4. Selection and optimization of simple sugars with process pH using *Enterobacter aerogens* for bioenergy options
5. Experimental assessment and optimization of industrial waste using *Enterobacter aerogens*: Integration for bioenergy production and treatment options
6. Kinetic analysis to assess the effect of bacterial growth and substrate utilization on bioenergy (hydrogen and methane) production options
7. Conclusions and Future Recommendations

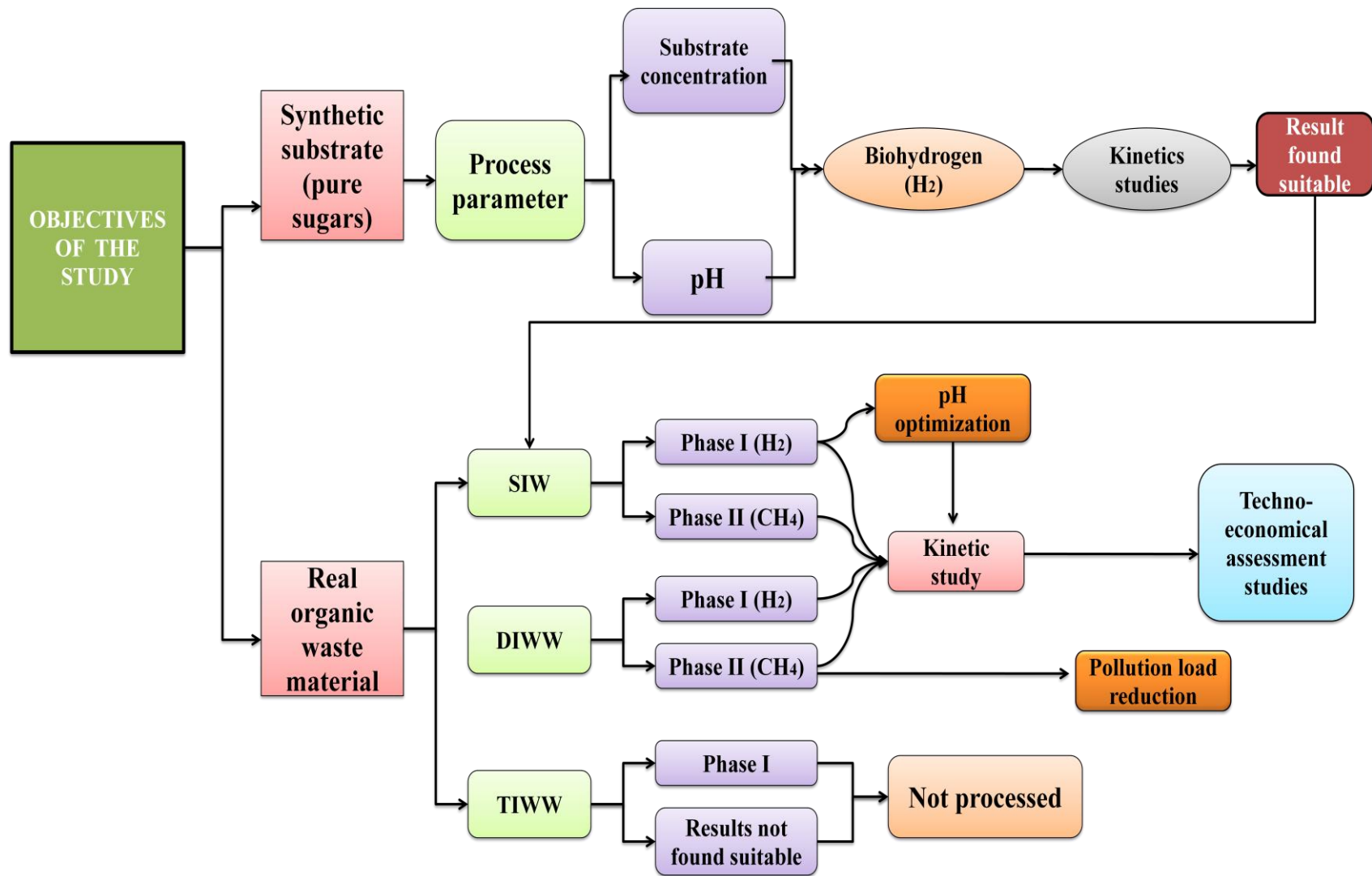
The chapter wise description of the work is as under:

Chapter 1: Introduction

In this chapter, an overview of the various available sources and processes for bioenergy options are discussed. The various processes for bioenergy production and feedstock/substrate compositions reviewed in particular to biogas, biofuel (bioethanol and biodiesel) and biohydrogen. Associated advantages and disadvantages with each are also discussed here to promote these at commercial level.

Chapter 2: Review of Literature

Literature review is broadly categorized into process route for fermentative hydrogen production using type of bacterial strains present in nature. Effects of various operational and process parameters are also discussed here to get optimized conditions for best efficiency in the process route. Operational parameters highlighted in the study with specific conditions are substrate and reactors type, whereas process parameters emphasised in this study are temperature, pH, volatile fatty acids, hydrogen partial pressure, enzymes and bacterial inoculums. Advance approach for bioenergy production options i.e. second stage process or two-stage approach is also reviewed here with their advantages and disadvantages at lab and pilot scale.



Graphical abstract for the study

*SIW- Sugar industry waste; DIWW- Dairy industry wastewater; TIWW- Tannery industry wastewater.

Chapter 3: Materials and Methods

This chapter focuses on the experimental methodology and analytical techniques used for each experimental plan. It outlines the basic information for microbial culture requirements and development for optimized conditions like type of substrate (sugar and industrial waste), effect of substrate concentration, effect of initial pH to assess the rate of biohydrogen production with experimental plans. Kinetic studies are also planned to assess the potential of experimental data in terms of production yield, specific bacterial growth (μ_m), biohydrogen production potential (P), rate (R_m) and lag phase (λ) using gas production models and substrate utilization models. So, this chapter is summarising the basic requirements needed to execute the experimental objectives in general and analytical/instrumental requirements to investigate the findings of Phase -I and Phase -II in particular.

Chapter 4: Selection and optimization of simple sugars with process pH using *Enterobacter aerogens* for bioenergy options

This Chapter deals with to find out the potential of pure simple sugars as the substrate, using facultative anaerobic bacteria *Enterobacter aerogens* for biohydrogen production. The study revealed that glucose is the most compatible and cost effective substrate, among all the selected sugars for the biohydrogen production with the yield of 0.87mol H₂ /mol of glucose consumed. The effect of process pH from initial start-up to final retention time was also analysed and the maximum biohydrogen production was found at the pH 5.5 for the process.

Study revealed that, there is a possibility to use of simple sugars and sugar based organic substrate materials like, organic residues and wastewater from industries for bioenergy production options. It also provides a suitable approach for clean environment in general and minimization of waste by bioenergy production approach in particular.

On the basis of these findings the selected bacterial strain *Enterobacter aerogens* was proposed for bioenergy production options (biohydrogen and methane) in integration with solid waste (organic residual waste) from sugar industry and liquid waste (i.e. wastewater) from dairy industry and tannery industry as the substrate.

Chapter 5: Experimental assessment and optimization of industrial waste using *Enterobacter aerogens*: Integration for bioenergy production and treatment options

This chapter is one step further in respect of previous one, here it is divided in three subsections on the basis of waste selected for study and emphasized on the use of these potential industries waste viz; sugar industry (solid organic residue), dairy industry (wastewater) and tannery industry (wastewater) for biohydrogen production with pure bacterial strain and further methane production from the fermented substrate. The selection of these industries for bioenergy production is based on their potential of waste generation, easy availability and previous studies on energy generation and waste treatment. The techno-economic benefits for bioenergy (hydrogen and methane) production is also evaluated in terms of waste assessment potential as a substrate for energy recovery and cost effectiveness to the other technologies in reference of bioenergy. Energy recovery from all selected industrial waste are also measured in terms of amount can be generated from per unit of substrate utilization and energy value of the produced energy.

5.1. A. Experimental set up plan-I (A): Assessment of sugar industry waste using *Enterobacter aerogens* for bio-energy (hydrogen and methane) production options with optimized parameters

The interactive effects of substrate concentrations (organic industrial residue) and pure bacterial strain for bioenergy production is investigated with cost effective assessment of this experimental plan with waste treatment and energy production approach simultaneously. From the results obtained at this part of chapter, it can concludes that utilization of organic residual substrate with pure strain can help to achieve a biohydrogen yield of 6.02 mM/g sugar with 40 g/l of substrate concentration has the highest gas production in Phase-I of the study. It has been also observed that the optimization of initial pH gave better results with pH 5.5 but growth of bacterial biomass is observed maximum with pH 6.5. The fermented substrate of the Phase -I was remained as soluble fermentation products in the form of solvents such as acetate, propionic acid, butyric acid and ethanol due to low substrate conversion efficiency in hydrogen production process These fermented substrate is further used for methane production and methane yield of 31 ml CH₄/ g sugar reduced with 40 g/l of fermented waste was found.

The maximum yield of bio-energy (biohydrogen and methane) production on per day basis was found in remarkable numbers with the present study (ESP-I, Phase-I and II). The obtained values of economic profit and energy recovery for bioenergy yield are also higher than the values obtained in double stage anaerobic digestion process as reported by other researchers.

5.2. B. Experimental set up plan-II (B): Assessment of dairy industry wastewater using *Enterobacter aerogens* for bio-energy (hydrogen and methane) production and treatment options simultaneously

In this experimental study of ESP-II, optimized substrate concentration was explored with microorganism *Enterobacter aerogens* for production of biohydrogen in phase I and methane in phase II. The study concludes that efficient success of the hypothesis i.e. bioenergy (hydrogen and methane) production and pollutants reduction from industrial wastewater. The best suitable optimum concentration, 75% of dairy industry wastewater was found feasible for bioenergy production with biomass growth as well as simultaneously pollutants reduction results for efficient treatment system such as higher efficiency achieved for phosphate (59% to 86%), sulphate (62% to 100%), nitrate (52% to 76%) at 25%, 50%, 75% and 100%. Similarly, maximum gas production was also achieved at 75% concentration of wastewater with 64% reduction in COD from initial to final HRT for experimental plan.

The maximum yield of bioenergy (biohydrogen and methane) production was found 0.562 L- H₂/ L DIWW, 0.59 L-CH₄./L-FDIWW in the present study (ESP-II, Phase-I and II). The obtained values of economic profit and energy recovery for bioenergy yield are higher than the values obtained in double stage anaerobic digestion process as reported by Park *et al.*, (2009).

Energy recovery from dairy industry wastewater was also measured in terms amount can be generated from per unit of substrate utilization and energy value of the produced bioenergy (MJ/day) provides a sustainable approach for utilization of industrial wastewater for bioenergy production with low cost of system.

5.3. C. Experimental set up plan-III (C): Assessment of tannery industry wastewater using *Enterobacter aerogens* for bio-energy (hydrogen and methane) production options

On the basis of findings, the energy production from tannery industry wastewater could not proceed may be the presence of high value pollutants concentration and

maximum toxicity is due to the presence of chromium ions which inhibit the growth of bacteria. But pretreatment of substrate and maintenance of anaerobic conditions can provide a better option for bioenergy production with tannery industry wastewater.

Therefore, this chapter finally concludes that (a) to achieve the best hydrogen yield an optimum substrate concentration should be obtained, which would be depending upon the substrate utilization capacity of the bacterial strain, (b) low and high pH variations directly affects the bacterial growth but improvement in biohydrogen yields only can attained through optimized process parameters, (c) pretreatment of substrate and maintenance of anaerobic conditions increases the cost of biohydrogen production but it seems to be cost effective and energy efficient at large scale production. Thus, two phase bioenergy production process provide enhanced COD reduction with hydrogen and methane as a product output for all the selected industrial waste materials (solid and liquid), and (d) Techno-economic assessment of energy yield (biohydrogen and methane) and economic bioenergy profit with both selected waste materials provides a better results with sugar industry waste in comparison to dairy industry waste as a substrate for bioenergy options with pure bacterial strain.

Chapter 6: Kinetic analysis to assess the effect of bacterial growth and substrate utilization on bio-energy (hydrogen and methane) production options

In view of the above said kinetic results, this chapter reviewed and analysed the trends of growth of bacteria, bioenergy (biohydrogen and methane) production options and substrate utilization kinetics by the application of different kinetic models. All kinetic models were critically analysed for all parameters taken for study. Following conclusions have been made from the thesis in particular:

- The logistic model applied for the growth of bacteria evaluated the maximum specific bacterial growth rate for sugar industry waste (SIW) and dairy industry wastewater (DIWW) at 40 g/l (0.36 h^{-1}) and 75% (0.52h^{-1}) concentration, respectively.

- Initial process pH on bacterial growth showed specific growth rate at 6.5 and most significant value (0.30 h^{-1}) in respect of biohydrogen production was found with initial pH of 5.5.
- The kinetics for bioenergy production evaluated in terms of biohydrogen and methane. Typical cumulative biohydrogen production curve fitted by the modified Gompertz equation using sugar industrial waste was found most significant (0.9981) with a substrate concentration, 40 g/l. Whereas, for dairy industry wastewater, 75% concentration shows most significant (0.9981) results in integration with *Enterobacter aerogens*. Similar trends of observations were found for both industrial wastes with of same concentrations for methane gas production for regression coefficients.
- Among the various selected models for kinetic studies, regression coefficient (R^2) was best found 0.98 with Moser model from sugar industry waste (molasses) and Hanes model was found best with R^2 value of 0.95 from dairy industry wastewater by selected bacterial strain.

Chapter 7: Conclusions and Future Recommendations

The results obtained are encouraging and study concludes that use of industrial waste materials (solid and liquid) provides a feasible solution with potential gas production in respect of clean energy source. It was also concluded that process parameters viz. substrate concentration and initial pH are very important for the efficient process mechanism. It was also found that industrial wastewater from selected industries can also be treated simultaneously with energy production options. Similarly, kinetic studies are performed with experimental findings also support the approach of sustainable solutions. The work done in thesis presents an answer (at least partially) to the problems associated with the clean and efficient production of bioenergy options. The recommendations for future work are also given in the chapter.