



ARISTOTLE
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Waste Utilization for Biohydrogen Production Through Photobiological Methods (Review Study)

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Presentation Contents

- Hydrogen
- Bibliography Processing Methodology
- Biophotolysis
- Photo-fermentation
- Key parameters
- Experiments-Results from Bibliography
- Future Prospects
- Conclusions

Hydrogen (1)

- H_2 - The most common chemical element in the universe
- High heating value - High efficiency
- Carbon Free Fuel - Zero GHGs emissions
- Alternative solution in environmental problems



Hydrogen (2)

Electrochemical



-Electrolysis
-Photo-electrolysis
**-High Temperature
Electrolysis**

Biochemical



-Dark fermentation
-Photo fermentation
-Biophotolysis

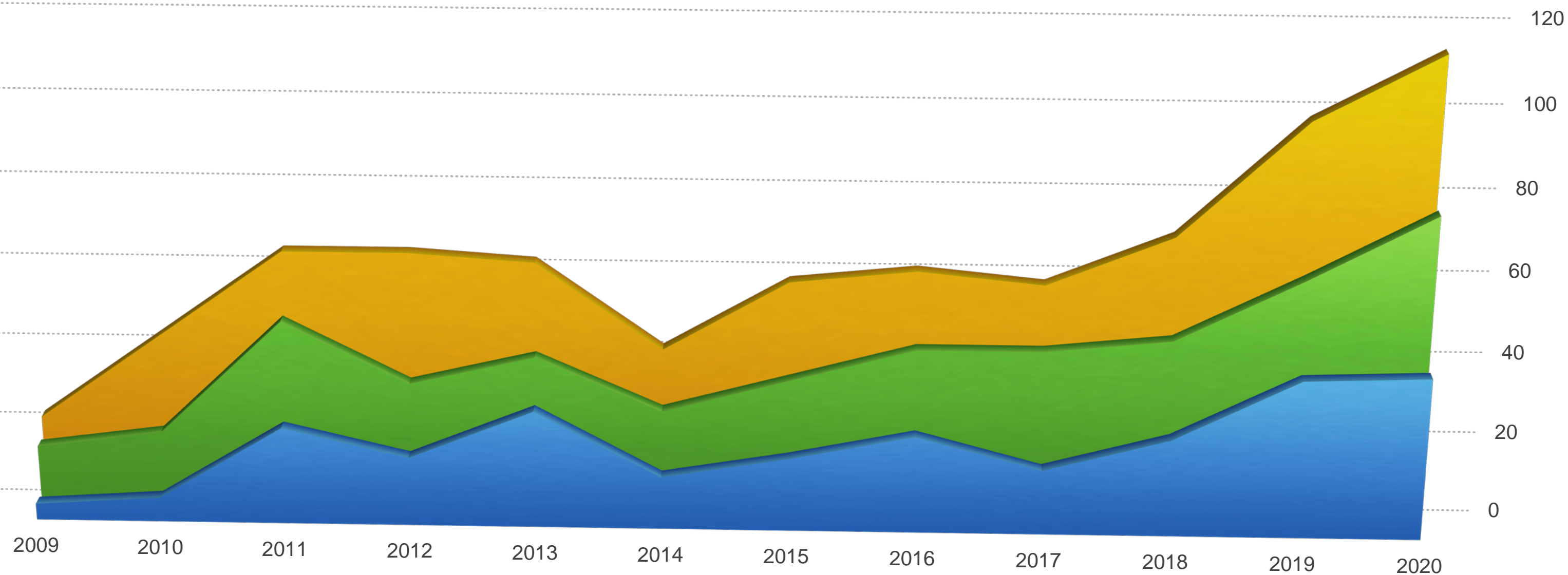
Thermochemical



-Gasification
**-Steam Methane
Reforming**
-Partial Oxidation

Literature Review

■ Photo-fermentation and Biophotolysis ■ Biophotolysis ■ Photo-fermentation

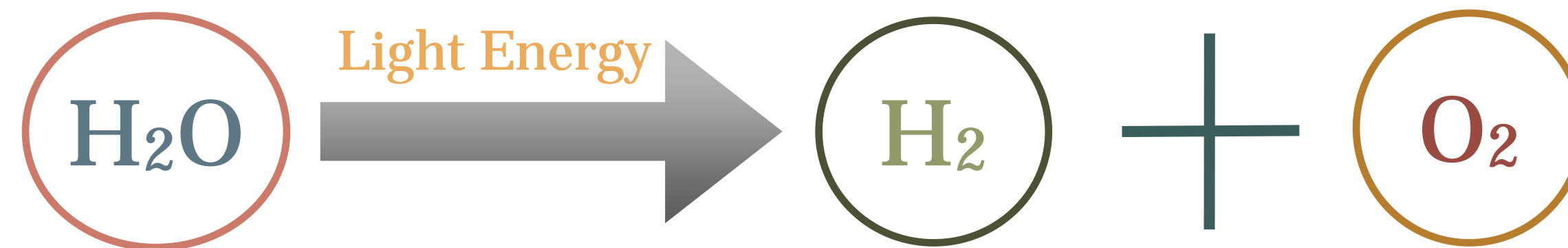


- Sources:
Science Direct - Scopus
- Rise in scientific interest in biohydrogen production methods
- Photo-fermentation attracts the greatest part of research



Biophotolysis

- Make use of the metabolic processes of microorganisms.
- Cyanobacteria - Microalgae (Anaerobic Conditions)



Two different methods

Direct Biophotolysis

Indirect Biophotolysis



Photo-fermentation

- Make use of the metabolic processes of PNS bacteria



- Carbon source : Organic Acids - Different types of organic wastes
- PNS bacteria catalyze the reaction
- Anaerobic Conditions



Process Parameters

- Carbon Source (Substrate)
- Photobioreactor Design
- Operation Mode
- pH
- Temperature
- Light illumination-intensity
- C/N ratio
- Nutrients-Inhibitors



Bioprocesses
Polyparametric system

Technical Parameters

- Substrate
 - Organic Acids (Acetate, Butyrate, Malate)
 - Organic originated wastes - sustainable alternative
- Photobioreactor
 - Open System - Continuous Operation
 - Closed System - Different types of operation
 - Batch - Widely used in lab experiments
(Easily Monitored Conditions)
 - Continuous mode - Favorable in large scale
(Low energy-operation costs)



Conditions (1)

* pH: Acidophilic microorganisms

Bacteria/Algae	pH range	References
Clostridium Species	5-6	G. Kumaravel Dinesh et al 2018
Rhodobacter sphaeroides	6,8-7,2	Shiladitya Ghosh et al 2016
Chlamydomonas reinhardtii	7	Shitralkha Nag Dasgupta et al 2010
Cyanobacterium Synechocystis and Cyanobacterium Gleocapsa	6,8-8,3	Kenzhegul Bolatkhan et al 2019
Oleaginous Microalgae	6,8	Dennapa Sengmee et al, 2016

* C/N ratio:

- Important factor for nitrogenase enzyme and PNSB's.
- N_2 - Anaerobic conditions maintenance.
- N_2 - Process Inhibitor (Nitrogenase).

Optimally



C/N = 25

Conditions (2)

* Temperature:

- Controls the metabolic processes (path) of the enzymes.
- Range of different microorganisms - Different optimal temperature.

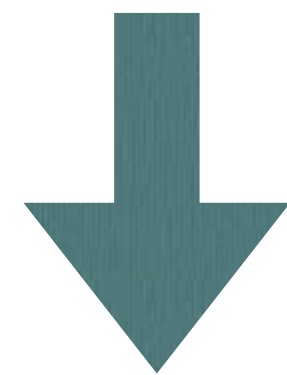
	Bacteria/Algae	Temperature (°C)	References
Biophotolysis ←	Microalgae	20-30	Ela Eroglu and Anastasios Melis, 2016
	Cyanobacteria	25-55	Shitrlekha Nag Dasgupta et al 2010, M.Y. Azwar et al, 2013
Photo-fermentation ←	PNS bacteria	25-35	Ming Foong Tiang et al, 2020

Conditions (3)

* Light illumination:

- Energy supply in the form of ATP.
- Natural / Artificial Source
- Light intensity \uparrow - Biohydrogen Productivity \uparrow

Until



Saturation Point

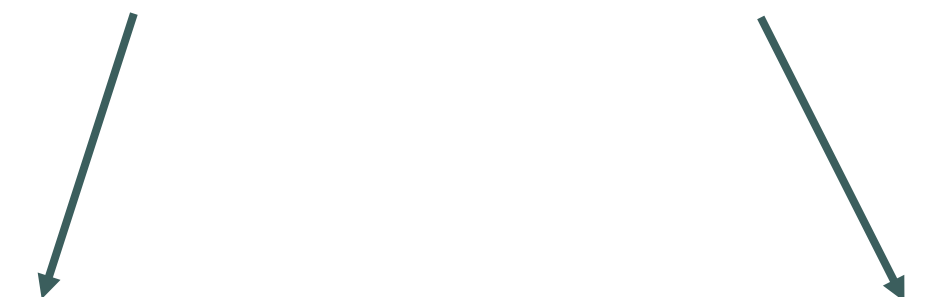
PNS bacteria



4000 - 5000 lux

Visible + UV

Biophotolysis



50 - 200 $\mu\text{E}/(\text{m}^2 * \text{s})$

Low conversion
Efficiency



Nutrients - Inhibitors

Chemicals	Improving	Inhibiting	Chemicals	Improving	Inhibiting
Iron	✓	-	Ethanol	✓	✓
Molybdenum	✓	✓	NaCl	✓	-
Nickel	✓	✓	Nano Ti-0 ₂	✓	-
EDTA	✓	✓	Methanol	-	✓
Vitamins	✓	-	Cooper ions	-	✓
Buffer Solutions	✓	-	Sulfide ions	-	✓
Magnesium	✓	-	Yeast	✓	✓

Experimental Data (1)

- Data presentation for biohydrogen production from microalgae and cyanobacteria

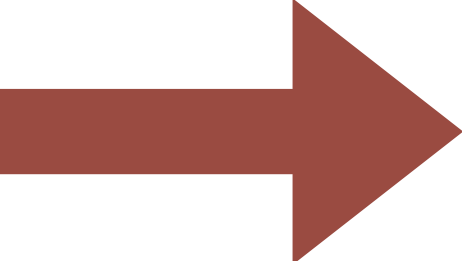

		Strain	Light Intensity	Temperature	pH	Biohydrogen production rate	Reference
Microalgae		Chlamydomonas MGA 161	115 $\mu\text{E} / \text{m}^2 / \text{s}$	30°C	8	4,48 ml / (L * h)	M.Y. Azwar et al, 2013
		Chlamydomonas reinhardtii CC-124	140 $\mu\text{mol photons} / (\text{m}^2 * \text{s})$ - both sides	28°C	7,2	7,5 ml / (L * h)	Ela Eroglu and Anastasios Melis, 2016
		Chlamydomonas reinhardtii 137c	110 $\mu\text{E} / \text{m}^2 / \text{s}$	25°C	7,2	2,5 ml / (L * h)	M.Y. Azwar et al, 2013
Cyanobacteria		Chlamydomonas reinhardtii CC-1036	120 $\mu\text{mol photons} / (\text{m}^2 * \text{s})$	27-29°C	7	9,2 ml / (L * h)	Ela Eroglu and Anastasios Melis, 2016
		Synechocystis sp. PCC 6803	90 $\mu\text{E} / \text{m}^2 / \text{s}$	30°C	-	18,4 $\mu\text{l} / (\text{mg Chl} * \text{h})$	Bekzhan D. Kossalbayev et al, 2020
		Anabaena cylindrica	140 $\mu\text{E} * \text{m}^{-2} * \text{s}^{-1}$	-	30 ml / (L * h)	S.N.A Rahman et al, 2016	Anabaena cylindrica
		Anabaena variabilis ATCC 29413	110-120 $\mu\text{E} / \text{m}^2 / \text{s}$	30°C	14,9 ml / (L * h)	M.Y. Azwar et al, 2013	Anabaena variabilis ATCC 29413

Experimental Data (2)

- Data presentation for biohydrogen production from PNS bacteria.

Bacterial Strain	Substrate	Process	Light Intensity	Biohydrogen production rate / yield	Reference
Rhodopseudomonas palustris WP3-5	Butyric acid	Batch	$135 \mu\text{E} * \text{m}^{-2} * \text{s}^{-1}$	24,9 ml / (L*h)	S.N.A Rahman et al, 2016
R. sphaeroides ZX-5	Lactate	Batch	-	103 ml / (L*h)	Xu Li et al, 2009
R. sphaeroides ZX-5	Butyrate	Batch	-	118 ml / (L*h)	Xu Li et al, 2009
Rhodopseudomonas faecalis RLD-53	Acetate	Batch	$150 \text{ W} / \text{m}^2$	36,60 ml / (L*h)	S.N.A Rahman et al, 2016
Rhodobacter sphaeroides ZX-5	Malate	Batch	$68 \mu\text{E} * \text{m}^{-2} * \text{s}^{-1}$	102,33 ml / (L*h)	S.N.A Rahman et al, 2016
Rhodobacter capsulatus ST410	Malate	-	$66 \text{ W} / \text{m}^2$	100 ml / (L*h)	Shitralekha Nag Dasgupta et al 2010
Rhodobacter sphaeroides KD131	Succinate	Continuous	-	2,3 mol / mol substrate	M.Y. Azwar et al, 2013
Rhodovulum sulfidophilum P5	Glucose	Batch	$100 \text{ mmol photons} / (\text{m}^2 * \text{s})$	7,07 mol / mol substrate	Bibi Shahine Firdaus Boodhun et al, 2017

Waste Utilization

- Waste utilization for biohydrogen production  Circular Economy Approach
- PNS bacteria can utilize multiple substrates  Organic Wastewater
- Reduce waste treatment - disposal costs.
- Pretreatment of the food and manufacturing industry wastewaters is necessary (dilution, filtration, pH neutralization, sterilization).
- Continuous processes are feasible and favorable.



Waste Utilization

- Wide range of waste appropriate (solid waste - wastewater - sewage sludge).
- Dark colored wastes reduce light penetration.

- Soy sauce wastewater
- Brewery wastewater
- Dairy wastewater
- POME combined with paper and pulp mill effluent
- Olive mill wastewater
- Sugar beet molasses
- Blackstrap molasses
- Tofu wastewater
- Palm oil, pulp and paper mills effluents
- Sugar refinery wastewater



Waste Utilization

Substrate (waste water or foodwaste)	Bacterial Strain	Pretreatment method	pH	Temperature	Light intensity	Biohydrogen Production yield	Reference
Soy sauce wastewater	Consortium of PNSB dominant strain: Rhodobium marinum	Autoclaving, dilution, pH neutralization	7	30	-	2,67 L H ₂ / L	Shiladitya Ghosh et al, 2016
Brewery wastewater	R. sphaeroides O.U.001	Filtration and sterilization	6	-	116 W / m ²	2,2 L H ₂ / L	Karen Trchounian et al, 2017
Brewery wastewaters	Rhodobacter sphaeroides O. U. 001	-	-	-	116 W / m ²	2,24 L H ₂ / L	S.N.A Rahman et al, 2016
Dairy wastewater	Rhodobacter sphaeroides O.U. 001	Filtration, autoclaving, re-filtration, dilution	7-7-02	28 ± 2	-	8,6 L H ₂ / L	Shiladitya Ghosh et al, 2016
POME combined with paper and pulp mill effluent	R. sphaeroides NCIMB8253	-	-	-	-	14,438 ml H ₂ / ml	Pretty Mori Budiman and Ta Yeong Wu 2018
Sugar beet molasses blackstrap molasses	Rhodobacter capsulatus JP91	-	7	30	-	10,5 mol H ₂ / mol sucrose	Shiladitya Ghosh et al, 2016
Palm oil, pulp and paper mills effluents	R. sphaeroides NCIMB8253	-	-	30	7000 lux	496 mL H ₂ / (L*h)	Karen Trchounian et al, 2017
Tofu wastewater	R. sphaeroides RV	-	-	-	8500 lux	4,32 L H ₂ / L	Ta Yeong Wu et al, 2012
POME combined with paper and pulp mill effluent	R. sphaeroides NCIMB8253	-	-	30	4000 lux	4,67 ml H ₂ / ml	Bibi Shahine Firdaus Boodhun et al, 2017
Blackstrap molasses	Rhodobacter capsulatus JP91	-	7	30	-	8 mol H ₂ / mol sucrose	Shiladitya Ghosh et al, 2016
Olive mill wastewater	R. sphaeroides O.U.001 ^{[1][5][9]}	-	7,2	32	200 W / m ²	39 ml H ₂ / L	Karen Trchounian et al, 2017
Sugar refinery wastewater	Rhodobacter sphaeroides O. U. 001	-	-	-	200 W / m ²	16,7 mol H ₂ / mol carbon	S.N.A Rahman et al, 2016

Future Prospects

Optimization of
cultivation techniques

Genetically modified
bacteria enzymes



Mixed
cultures

Hybrid Systems

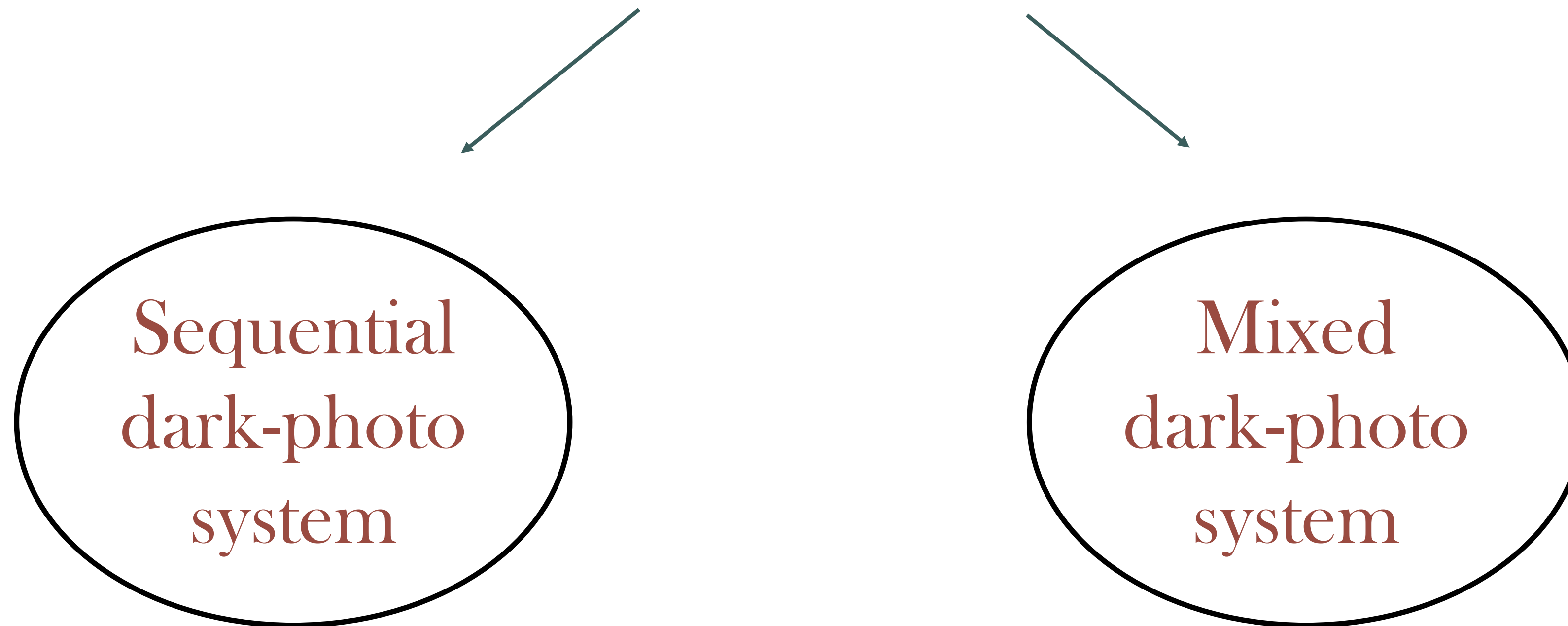
Advanced
photobioreactor's
design and operation

Hybrid Systems

- Dark - photo hybrid system.
- 1st step: Waste processing via dark fermentation.
Products: Biohydrogen + VFAs
- 2nd step: VFAs further utilization via photo fermentation.
- Improved biohydrogen production - conversion efficiency
- Utilization of VFAs (dark fermentation by-products).
- Increase in working process time.

Dark-Photo System

Two different process paths



Conclusions

- Main advantages:
 - Cheap - accessible microorganisms.
 - High thermodynamic efficiency (theoretically).
 - Nitrogen Fixation – Environmental benefits
 - Waste utilization - Circular economy approach.
- Main Drawbacks:
 - Oxygen Sensitive microorganisms + Oxygen as process byproduct.
 - Scale up operation difficulties.
 - High energy demands - Low efficiency.
 - Photobioreactor cost - specialization.

Conclusions

- Sunlight utilization - energy conversion into fuel (biohydrogen).
- Wide range of substrate utilization (wastes).
- Enzymes metabolic processes are crucial - anaerobic systems
- Genetic engineering - Biotechnology —————> Enzymes modification
- Hybrid dark - photo systems —————> Sustainable option
- Decarbonization - Green hydrogen production

Thank you for your attention!