POPULAR DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCQTION

AND SCIENTIFIC RESEARCH

KASDI MERBAH OUARGLA UNIVERSITY

Faculty of applied sciences.

Department of process engineering.

Thesis

ACADEMIC MASTER

Domain: Science and Technology

Sector: Industrial and petrochemical

Specialty: Refining Engineering

Presented by:

KHOUDRANE Nesrine, and ZALANI Rayane.

Theme:

Contribution to the study of natural alternatives for chemical activator to amino acids

Presented Publically on: 18.06.2025

Before the jury:

Mr. GOUDJIL Bilal	**Professor (UKM Ouargla)**	**President**
Mrs. CHAIB Hadjira	**MCA (UKM Ouargla)**	**Examiner**
Mrs. ZIGHMI Souad	**MCB (UKM Ouargla)**	**Supervisor**

Academic Year: 2024/2025

Acknowledgments

All praise is due to Allah, whose grace has guided us to the completion of this humble work.

We extend our heartfelt gratitude to our supervisor Dr. Souad Zighmi, for her patient guidance.

We extend our sincere thanks to the members of the jury GOUDJIL

Bilal and CHAIB Hadjira, ZIGHMI Souad for agreeing to

referee this work.

We would like to thank Bilal Ben Abidi for his advice and guidnece and help during this work.

We are grateful to the Process Engineering Department and to Kasdi Merbah University for providing an inspiring academic environment.

Finally, we thank Allah once more and pray that this work proves beneficial to science and society.

Dedication

Above all, praise be to ALLH who gave us strength.

To my beloved parents, Azzedine and Amel, whose endless love, sacrifices, who gave me everything I needed to pursue my studies, also my sister Lamia and all my siblings, whose love continually lifts me up.

To my dear friends—Nadjet haloui, Sounds Toumi, and to my closest friend Amira touil the companions who shared every high and low of university life.—your presence made every day brighter.

This thesis is for every single person who believed in me and encouraged me along the way.

Nesrine khoudrane

Dedication

Firstly my dear parents father and mother the school of my childhood, and i tell them dad and mom

no dedicates and words are sufficient to describe my love and my respect for you.

Secondly my 3 little sisters l wish them all the best joy and success in their lifes.

Inshallah l will lift your spirits and make you dear father and mother very proud of me.

Rayane zalani

الملخص :

تهدف هذه الدراسة النظرية والتجريبية الى استخدام انزيمات طبيعية مستخرجه من الطحالب لازاله ثاني اكسيد الكربون في عمليات التكرير، تم تطبيق بروتوكولين، واظهر الثاني نتائج افضل من حيث نقاء الانزيم وامتصاص ثاني اكسيد كربون سجلت طحالب مثل كلادوفورا و أولفاسي مردودية وصلت الى 96٪ مما يثبت فعاليتها كحل بيئي.

الكلمات المفتاحية: امتصاص ثنائي اكسد الكربون ، استخراج الطحالب، إنهيدراز الكربونيك، التكرير الأخضر.

Abstract

This theoretical and experimental study explores natural alternatives for CO_2 removal in refining using carbonic anhydrase enzymes extracted from local algae in Algeria. Two protocols were tested, and results showed that Protocol 2 offered better enzyme purity and CO_2 absorption. Algae such as Cladophora and Ulvaceae achieved yields up to 96%, proving the potential of bio-based solutions.

Keywords: CO_2 capture, algae, enzyme extraction, carbonic anhydrase, green refining.

Résume

Cette théorique et expérimental étude examine des alternatives naturelles pour la capture du CO_2 dans le raffinage à travers l'utilisation d'enzymes extraites d'algues locales. Deux protocoles ont été testés, et le second a montré une meilleure pureté enzymatique et absorption du CO_2. Certaines algues comme Cladophora et Ulvaceae ont atteint un rendement de 96 %, confirmant leur efficacité.

Mots-clés : Capture de CO_2, algues, extraction enzymatique, anhydrase carbonique, raffinage vert.

List of indices and abbreviations

CO_2: Carbon Dioxide.

CA / Cas: Carbonic Anhydrase / Carbonic Anhydrases.

CCUS: Carbon Capture, Utilization and Storage.

CCS: Carbon Capture and Storage.

CCC: Cryogenic carbon capture.

MEA: Monoethanolamine.

DEA: Diethanolamine.

MDEA: Methyldiethanolamine.

MMMs: Mixed matrix membranes.

AMP: 2-Amino-2-Methylpropanol.

DGA: Diglycolamine.

DIPA: Diisopropanolamine.

NaOH: Sodium Hydroxide.

KH_2PO_4: Potassium Dihydrogen Phosphate.

K_2HPO_4: Dipotassium Hydrogen Phosphate.

HCO_3^-: Bicarbonate Ion.

DW: Dry Weight.

ILs: Ionic Liquids.

List of tables

List of Figures

GENERAL INTRODUCTION

General introduction

There is a growing demand for optimized energy systems due to the accelerating pace of global industrial development. Oil refining remains the backbone of the world's energy supply chain. However, this sector is also one of the largest contributors to anthropogenic carbon dioxide (CO_2) emissions, highlighting the urgent need for effective and sustainable CO_2 removal solutions **[1].**

Traditional post combustion carbon capture techniques, and especially those employing amine-based chemical solvents, such as monoethanolamine (MEA), are well established. However, they are accompanied by several major disadvantages such as excessive energy consumption and toxicity as well as solvent degradation and corrosivity **[2].**

In recent years, research has focused on natural bio-based approaches with the promise of being more environmentally friendly and sustainable. Among these, amino acid, in particular algal amino acids have gained much attention because they can be degraded, less-volatile and reacts with CO_2 **[2].**

Additionally Enzymatic systems, such as carbonic anhydrase (CA), have demonstrated the ability to catalyse the hydration of CO_2 at rates that exceed quite significantly the uncatalysed hydration reaction, making them attractive biocatalysts for carbon capture applications **[2].**

Both microalgae and macroalgae are abundant, renewable, source of bioactive compounds such as amino acids and CA enzymes. Their fast growth, adaptability to marginal lands, and tolerance to industrial CO_2 rich flue gases make them promising candidates for integration into carbon capture systems.

The use of algal biomass in developing green solvents and bio-enhanced CO_2 absorption processes aspire the interconnection between environmental preservation and industrial feasibility **[3].**

This thesis investigates the possibility of using amino acid and enzyme extracts from algae as natural activators for CO_2 absorption in decoking solutions. The book is divided into two main parts:

- ✓ A theoretical review of conventional CO_2 capture methods alongside bio-based alternatives such as amino acids, biopolymers, and carbonic anhydrase systems.
- ✓ A practical section focused on enzyme extraction from various Algerian algal species and their application in laboratory-scale CO_2 capture experiments.

Through the comparison of conventional and biological means, this research seeks to elucidate the viability of biotechnology using algae for efficient, affordable, and ecologically friendly CO_2 capture. Combining process engineering with biotechnological has the potential to present an innovative solution for the reduction of emissions both ecologically and economically **[4].**

CHAPTER I:

ADVANCED CO_2 CAPTURE STRATEGIES IN REFINING ENGINEERING

Chapter I: Advanced CO2 capture strategies in refining engineering

I. 1 Introduction:

Refining is one of the major contributors to meeting global energy demands. However, like many other sectors, it also contributes a significant portion of greenhouse gas emissions – particularly CO_2. Refining contributed approximately 1.4 billion tons of CO_2 emissions in 2019, a large proportion of world emissions. The United States contributed about 18% of that total. Combined with the extraction of oil and gas, refining represents approximately 15% of energy-related emissions worldwide around 5.1 billion tons of CO_2.

Due to these environmental concerns, strict regulations have been implemented to mitigate emissions of CO_2 from manufacturing processes: the U.S. Environmental Protection Agency has established emission limits and has provided funding for cleaner technologies, while at the state level state initiatives have supported industrial plants that participate in decarbonization strategies **[1-3].**

Carbon Capture, Utilization, and Storage (CCUS) technologies are promising and can capture as much as 90% of CO_2 emissions from refineries. Create efficiency and scalability: the importance of refining engineering methods and carbon capture technology advances this chapter will focus on traditional CO_2 capture methods in refining engineering and their environmental impact **[4].**

I. 2 Existing CO_2 Capture Technologies in Refining:

I. 2. 1 Amine-Based Absorption:

Amine-based CO_2 capture is the most widely used chemical absorption process in refinery and industrial applications. It involves scrubbing CO_2 from flue gases using aqueous amine solutions, most commonly monoethanolamine (MEA). The basic chemical reaction involved is: $CO_2 + 2RNH_2 \rightleftharpoons RNH_3^+ + RNHCOO^-$

(Formation of carbamate from amine and carbon dioxide)

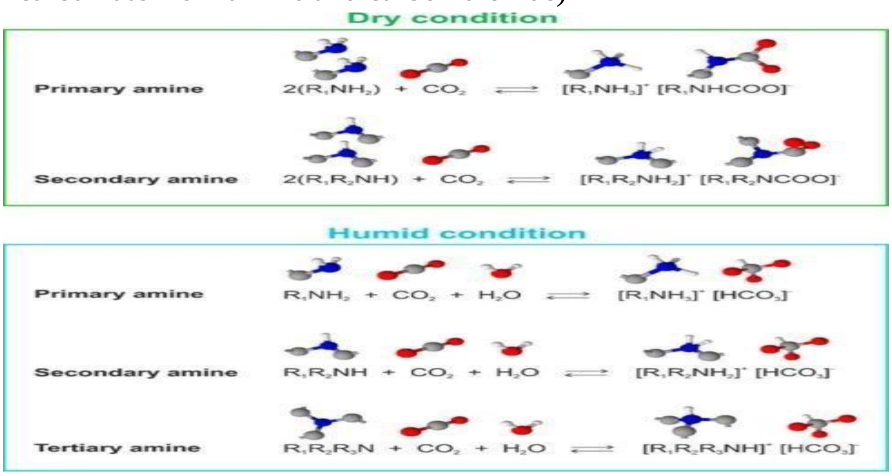


***Figure I-1:** Examples of reaction schemes of CO2 adsorption under dry and humid conditions.*

I. 2. 2 Advantages:

- High Capture Efficiency: MEA and other primary amines can remove up to 90–95% of CO_2 from flue gases.
- Industrial Maturity: This technology is commercially available and has decades of operational data.
- Solvent Regeneration: CO_2-loaded amines can be regenerated through heating, making the process cyclic and reusable **[5].**

I. 2. 3 Challenge:

- High Energy Demand: Solvent regeneration requires significant heat, usually via steam, increasing overall energy costs.
- Solvent Degradation: Amines degrade in the presence of oxygen and acidic impurities, producing heat-stable salts and reducing efficiency.
- Corrosion and Equipment Wear: The corrosive nature of amine solvents can damage pipelines and towers without proper mitigation.
- Environmental Risk: Amine emissions and degradation byproducts (e.g., nitrosamines) can pose health and ecological risks **[5].**

I. 3 Industrial Applications in Gas Treatment Units:

Amine-based gas treating (also commonly amine scrubbing) is widely used industrially for the removal of acid gases including hydrogen sulfide (H_2S) and carbon dioxide (CO_2) from several gas streams **[6].**

This is a core process associated with oil refineries, petrochemical plants and natural gas processing plants for improving product quality and to follow environmental regulations. In normal mode, the sour gas flows through an absorber tower from top to bottom contacting a downflowing aqueous amine solution typically MEA (20–30% w/w) in monoethanolamine pornostadua **[7].**

Amine reacts with acid gases chemically and these are regeneratable in a regenerator unit by heat so that the CO_2 is released from the compound that set it into absorption phase but the amine should be regenerated for using it again through the application heat in the regenerator unit **[4].** Although very effective this process suffers from the problems of high energy required for solvent regeneration, solvent degradation and equipment corrosion. Alternative solutions, including hybrid systems that combine amine solvents with ancillary technologies (e.g. membranes and solid sorbents) are under development to increase efficiency and decrease operational costs to solve the aforementioned problems **[8,9].**

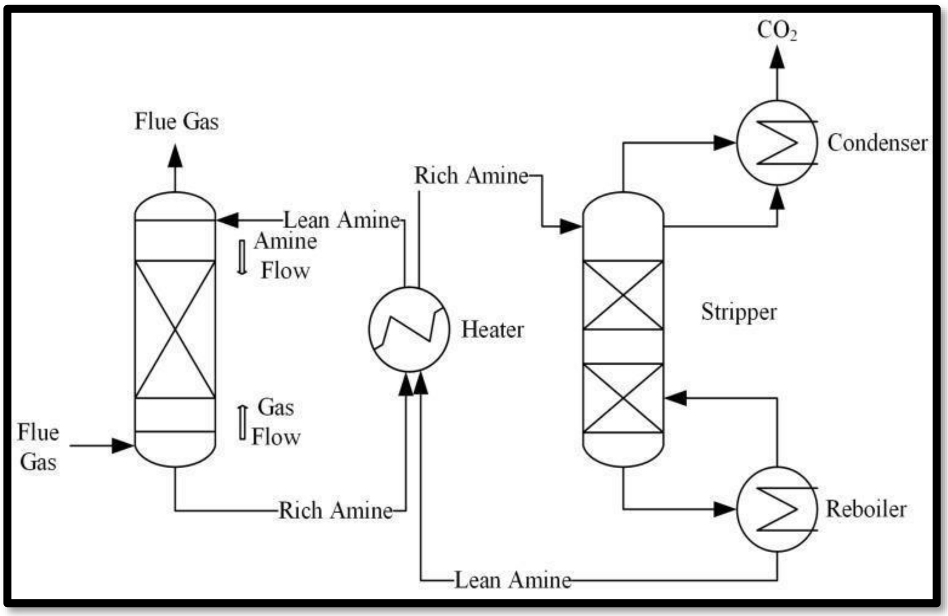


Figure I-2: Process flow diagram of amine-based CO_2 capture in industrial gas treatment units.

I. 3. 1 Energy Consumption and Environmental Concerns:

- **Energy Consumption :**

Regeneration of amine solution, especially MEA (Mono Ethanol Amine), is a high thermal energy process. Research has demonstrated that regeneration of 30 wt% MEA solution is around 3.6 GJ per tonne of CO_2 captured or equivalently 70–80 % of the cost in CO_2 capture process is associated with desorption (approximately 3.6 to 3.8 GJ per tonne of CO appro Captured). This need for high levels of energy arises from the necessity to break the chemical binding between CO_2 and amine resulting from absorption, as well as to heat the solution to required desorption temperatures. Therefore this decrease the efficiency of the power plants as a whole and increase the maintenance cost **[10].**

- **Environmental Concerns:**

Degradation of Amine is a serious concern with the result formation of environmental hazardous by-products. Amines degrade in oxidative and thermal pathways that occur during CO_2 capture, which causes the formation of ammonia, aldehydes, and nitrosamines **[11].**

Uncontrolled disposal of these degradation products will make them being environmental pollutants. The amine solution is also extremely aggressive and requires a construction of capture facilities from corrosion-resistant materials. Impurities like SO_2 and O_2 in the flue gas can increase this corrosion, as well which will have to be compensated with solvent degradation increase and maintenance requirements, and costs. Solutions to these challenges are key in order for amine-based CO_2 capture technologies be implemented in a sustainable and thus economicalmanner **[11,12].**

I.3.2. Membrane Separation Technology:

Membrane separation technology proves to be a good choice for CO_2 capture in the refining industry, as it saves energy and can be easily installed within current equipment. Compared to older methods that utilize amines in order to separate CO_2 from flue gases, membranes can effectively separate CO_2 without having any chemical reactions. This method is much less energy intensive, and less costly when dealing with solvent degradation. Development in improving membrane materials which include polymer membranes, mixed matrix membranes (MMMs) and inorganic membranes have dramatically increased performance especially in high temperatures and pressures **[13].**

One good example is a membrane system designed to capture CO_2 at the time of combustion that has been proven to work well (and can now be used large scale for facilities in factories), and more recently updates in all-carbon membranes have shown that they continue to perform well, even if we consider difficult problems (water vapor and hydrocarbon impurities) **[13,14].**

I. 3. 3 Cryogenic and Oxyfuel Combustion Techniques:

Cryogenic carbon capture (CCC) and oxyfuel combustion are two emerging technologies under evaluation for CO_2 separation in refinery and industrial contexts.

- ✓ **Cryogenic Carbon Capture (CCC):** This method cools the gas stream to extremely low temperatures (below -135°C), where CO_2 condenses and is separated as a liquid or solid.
- ✓ **Oxyfuel Combustion:** Involves burning fuel in nearly pure oxygen instead of air, resulting in flue gases primarily composed of CO_2 and water vapour making CO_2 separation easier **[15].**

I. 3. 4 Advantages:

High CO_2 Purity: Both methods yield high-purity CO_2 (>95%), reducing the need for further purification.

- ✓ **No Chemical Solvents Required:** Avoids degradation issues related to amine-based solvents.
- ✓ **Lower Environmental Emissions:** Especially for oxyfuel, NOx and SOx formation is minimized due to reduced nitrogen input.
- ✓ **Recyclable Cooling:** In CCC, the cold energy can sometimes be partially recovered and reused, improving efficiency **[16].**

I. 3. 5 Challenges:

- **High energy demand:** cryogenic equipment requires a lot of energy to compress and cool the gas especially at refinery scales.
- **Infrastructure intensive:** Oxyfuel combustion requires high-priced air separation

units (ASUs) for purified oxygen.

- **Limited industrial maturity:** Both technologies are still in the pilot or demonstration stages and there has been little full-scale deployment in refineries.
- **Complexity of Operation:** Adding cryogenic and oxyfuel plants to existing plants requires extensive plant modifications **[15-17].**

I. 4 Environmental and Economic Impact of Traditional CO_2 Capture:

I. 4. 1 Environmental Risks of Amine-Based Absorption:

Specific environmental problems associated with monoethanolamine (MEA) systems in CO_2 capture include: Amine degradation in MEA systems generates unpleasant by-products including nitrosamines and nitramines, which can pollute atmospheres, groundwater and produce carcinogenic material. Disposal of amine contaminated waste presents environmental challenges since traditional methods such as combustion and landfilling might no longer be suitable **[18].**

I. 4. 2 Challenges with Membrane Separation and Cryogenic Techniques:

- **Membrane Separation Challenges:**

  - ✓ Exposure to membrane fouling which can decrease CO_2 permeability and system efficiency.
  - ✓ Material degradation over time, especially under high-pressure and chemical-rich conditions.
  - ✓ High replacement and maintenance costs especially in large-scale or long-term operations **[24].**
  - ✓ Selectivity trade-offs, where increasing permeability often reduces separation accuracy for CO_2 **[19-21].**

- **Cryogenic CO_2 Capture Challenges:**

  - ✓ High Energy Consumption: Cryogenic processes require significant energy input to achieve the low temperatures necessary for CO_2 liquefaction or desublimation, impacting overall efficiency.
  - ✓ Operational Complexity: Maintaining cryogenic temperatures demands advanced refrigeration systems and insulation, increasing operational complexity and costs.
  - ✓ Scalability Concerns: The infrastructure and energy requirements of cryogenic systems can limit their scalability, particularly in existing refinery settings where retrofitting may be challenging.

I. 4. 3 Economic Drawbacks of Traditional CO_2 Capture :

Traditional CO_2 capture methods require significant capital investment, as well as very high operating costs. Solvent regeneration in amine-based systems requires a large amount of energy and thus significantly increases operating costs. The design and building of the infrastructure required to transport and store CO_2 raises operating costs, thus preventing its widespread deployment in refining plants **[19].**

I. 4. 4 Bio-Based Approaches to CO_2 Capture in Refining:

Bio-based solutions are emerging as alternatives for CO_2 capture because traditional methods present economic and environmental challenges to the refining industry. Natural solutions represent sustainable and economical approaches for reducing carbon emissions **[6].**

I. 4. 5 CO_2 Capture Using Microalgae:

Naturally absorbing CO_2 and converting it into biomass, microalgae are photosynthetic microorganisms. In industrial facilities, CO_2 can be directly sequestered from flue gases using algae bioreactors **[21].**

I. 4. 6 Advantages:

- High Fixation Efficiency: Compared to terrestrial plants, microalgae can fix CO_2 at rates that are noticeably higher **[27].**
- Bio-product Generation: Bio-hydrogen, biodiesel, and other valuable products can be made from the biomass that is produced **[27].**
- Wastewater Treatment: By absorbing CO_2 and treating wastewater at the same time, microalgae can improve sustainability **[25].**

I. 4. 7 Challenges:

- Cultivation System Design: While closed photo bio-reactors are costly, open ponds are less expensive but more likely to become contaminated.
- Flue Gas Composition: Chemicals harmful to microalgae may be present in industrial flue gases, which could impact their growth and CO2 capture effectiveness.
- Operational Costs: Economic viability may be impacted by the high energy requirements for cultivation and harvesting processes. **[26].**

Example: Spirulina and Chlorella vulgaris strains are commonly used for carbon fixation in lab-scale studies and pilot industrial algae ponds.

I. 4. 8 Enzyme-Assisted CO_2 Capture:

- The enzyme carbonic anhydrase (CA) speeds up the transformation of CO_2 into bicarbonate which improves carbon capture systems' performance. Implementation of CA into CO_2 capture systems helps to cut down energy needs and elevates their performance. Researchers have recently studied the immobilization of CA to enhance its durability and enable its repeated use in industrial settings **[22].**

Example: Novozymes has developed carbonic anhydrase enzyme formulations that are being tested in post-combustion capture units.

I. 5 Comparative Summary of CO_2 Capture Technologies:

Table I-1: A comparative overview of various CO_2 capture technologies [8].

Technology	Capture Efficiency	Energy Requirement	Environmental Impact	Industrial Maturity	Scalability
Amine Absorption	90–95%	High (3.6–3.8 GJ/ton)	High (amine degradation)	Commercial	High (but costly)
Membrane Separation	0–90%	Medium	Low (no chemicals)	Commercial/Pilot	Moderate
Cryogenic Capture	>95%	Very High	Low (no solvent)	Pilot	Limited
Oxyfuel Combustion	>95%	High (oxygen production)	Very Low (low NOx/SOx)	Pilot	Moderate
Microalgae Systems	~50–80%	Low–Medium	Very Low (natural)	Early Pilot	Moderate (land use)
Enzyme Systems	85–90%	Low	Very Low	Pilot	Emerging

I. 6 Conclusion:

As environmental pressure grows on the refining industry to reduce its effect on the environment, it’s becoming clear that many of the conventional methods for capturing CO_2 aren’t very effective: Mono-ethanolamine (MEA) absorption and cryogenic separation have been able to help reduce CO_2 emissions, but there are downsides such as high energy consumption, expensive equipment and concerns about chemicals leaking back in.

That’s why more and more attention is being paid to bio-based solutions. Methods derived from nature allow for capture of CO_2 in ways that can be highly economical, clean, and sustainable. Whether it’s through living organisms, enzymes, or carbon-rich byproducts, bio- based technologies are opening up a new route into cleaner and more sustainable refining.

A solution in particular is showing some promise in the way that we think about CO_2 capture. In chapter two, we’ll discuss how this simple organism might be the answer to what might otherwise be a difficult problem (capturing CO_2 efficiently).

CHAPTRE II:

BIO-BASED CO_2 CAPTURE USING AMINO ACIDS AND ALGAE DERIVED SOLUTIONS

Chapter II: Bio-Based CO_2 Capture Using Amino Acids and Algae-Derived Solutions

II. 1 Introduction:

This chapter foucses specifically on bio-based amino acids more particularly those derived from algae as natural alternatives to traditional amine solvents for CO_2 capture applications. Due to their non-toxicity, renewability, and reactivity with CO_2, amino acids have attracted growing attention in industrial carbon capture applications **[28].**

Algae, as a compact and renewable biomass source, provide a sustainable pathway to the synthesis of amino acids that are biodegradable and thermally stable, which are amenable to aggressive refining conditions. Their usefulness also opens up the potential for combination with enzymatic or hybrid systems for enhanced absorption kinetics. By narrowing the focus, this chapter aims to evaluate the true potential of algae-derived amino acids as a sustainable solution for CO_2 removal in refining **[29].**

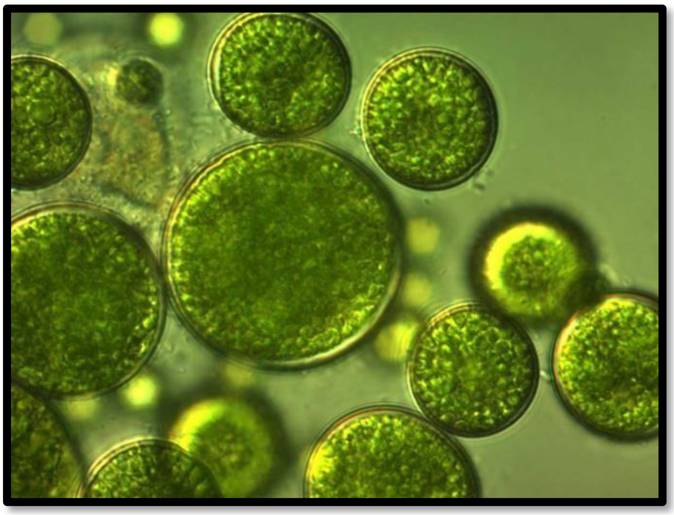


Figure II-1: algae as natural alternatives.

II. 2 Natural Occurrence of Amino Acids:

Amino acids are organic compounds naturally found across all living organisms plants, animals, microbials, and prominently in sea biomass like algae. Amino acids are structurally endowed with amine and carboxylic acid functional groups which enable them to react with CO_2 and therefore are potential carbon capture reagents. Nature's amino acids are produced by central metabolic routes like glycolysis and the citric acid cycle. Algae and cyanobacteria specifically have the ability to accumulate high levels of free amino acids like glutamate and glycine, particularly under stress or starvation conditions. Algae's high growth rate, tolerance to non-arable lands, and high nitrogen content make it a cost-effective and environmentally friendly source.

Because of their renewability, nontoxicity, and compatibility with CO_2, naturally occurring amino acids particularly those from algae are becoming prominent as potential alternatives to synthetic solvents in the technology of CO_2 capture **[30].**

II. 2. 1 Industrial Applications of Amino Acids:

Amino acids have historically been used in food, drug, and cosmetic industries, given their essential biological roles. In recent years, the potential of their application was also realized in environmental technologies, particularly carbon capture and storage (CCS).

In the refining and energy sectors, amino acids rise as natural absorbents of CO_2. Their amine groups react reversibly with carbon dioxide just as conventional amines would do, however, they present the benefits of being less volatile and toxic. For example, aqueous solutions of amino acids such as l-valine have shown to possess a good capacity for absorbing CO_2 at room temperature, thus providing a green alternative to conventional solvents **[31].**

Amino acid salt solutions, including potassium l-prolinate, show better reactivity towards CO_2 than their sodium counterparts. In addition, the solutions possess good physicochemical properties with respect to viscosity and density; thus, they are promising candidates for industrial applications in CO_2 capture **[31].**

Some operational advantages offered by amino acid-based solvents in CCS applications are:

- **Safety to Environment:** According to toxicity studies, these solvents are less toxic and hence can be biodegraded with minimum environmental impact.
- **Efficiency of Operation:** Solvents needing less energy for regeneration also lends to higher efficiency in the capture processes.
- **Equipment Longevity:** Reduced corrosion potential increases the lifespan of industrial equipment **[32].**

II. 2. 2 Types of amines used to capture carbon dioxide:

- monoethanolamine (MEA),
- nmethyldiethanolamine (MDEA),
- 2-Amino-2- methylpropanol (AMP),
- Piperazine (PIPA),
- diglycolamine (DGA),
- diethanolamine (DEA),
- ndi-isopropanolamine (DIPA) **[37].**

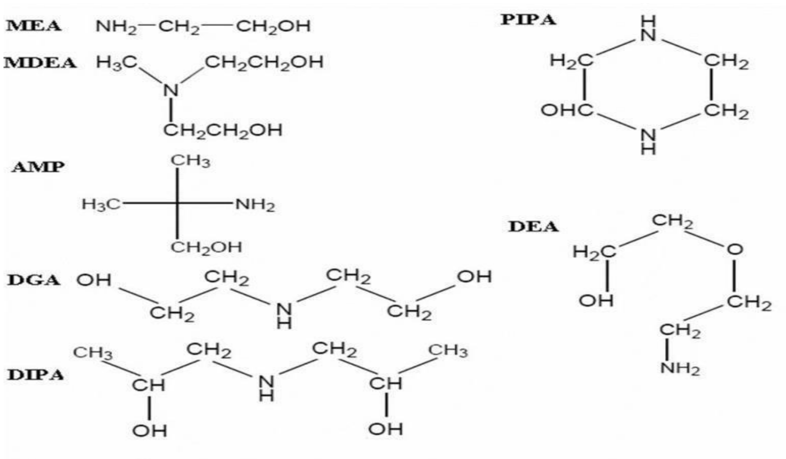


Figure II-2: Chemical structures of the amines most used in CO_2 capture.

II. 2. 3 Absorption Chemistry: Amino Acids vs. Traditional Amines:

The absorption of CO_2 through amino acids functions through comparable basic mechanisms as traditional amines yet the differences in chemical properties lead to substantial effects on operational performance and system sustainability and environmental impact.

➢ **Mechanism Comparison:**

- **Traditional Amines (such as MEA, DEA):** React with CO_2 primarily through carbamate formation. The reaction rate is quick yet it can be reversed with high energy needs of about 3.5 to 4.0 GJ per ton CO_2. The compounds undergo decomposition when exposed to oxidative and thermal conditions which generates toxic substances.
- **Amino Acids (such as Glycine, Sarcosine):** Form carbamates while demonstrating superior heat stability and limited evaporation characteristics. Their zwitterionic nature provides better resistance to oxidative decay as well as reduced effects on environmental systems **[33]**.

a. Advantages of Amino Acids:

- ✓ Non-volatile and biodegradable,
- ✓ Lower corrosiveness,
- ✓ High absorption capacity at moderate temperatures,
- ✓ Compatible with enzymatic enhancement (such as carbonic anhydrase) **[33]**.

b. Challenges:

- ✓ Higher viscosity in aqueous solutions,
- ✓ Slower reaction rates compared to MEA without enzymatic support,
- ✓ Precipitation at high concentrations (requires optimization) **[33]**.

Amino acids provide a sustainable replacement for standard amines while demonstrating equal CO_2 absorption capabilities especially when used in enzymatic or hybrid approaches **[33].**

II. 3 Algae as a Novel Source of Bio-Based Amino Acids:

The scientific community is increasingly recognizing algae both micro and macro types as valuable sources for sustainable amino acids because of their protein-rich content along with fast growth rates and minimal demands on land and freshwater resources. Algae provide a productive solution that serves two purposes by helping reduce CO_2 levels while supplying materials for bio-based carbon capture products **[80].**

II. 3. 1 Types of Algae for CO2 Capture:

a) ***Microalgae:*** Microscopic single-celled algae are extremely effective at absorbing CO2 because of their high surface area-to-volume ratio. Examples commonly Include Chlorellaand Spirulina **[21].**

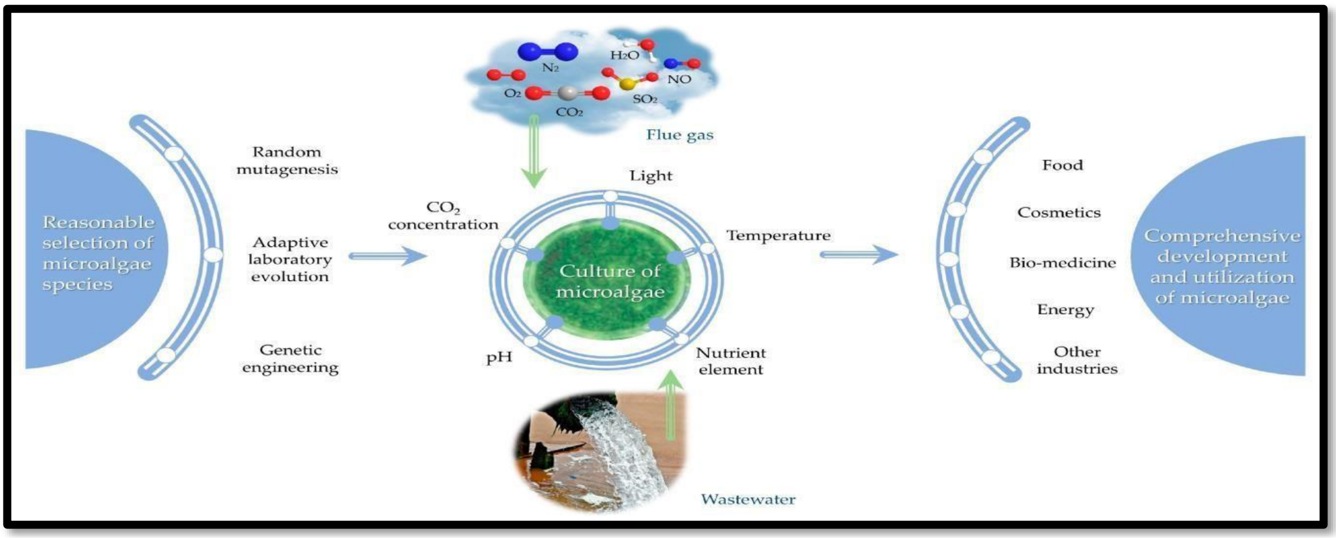


Figure II-3: The technical flow chart of microalgae carbon sequestration.

b) ***Macroalgae:*** Larger multicellular algae, such as seaweed, can be grown in open ponds or incorporated into existing aquaculture systems **[21].**

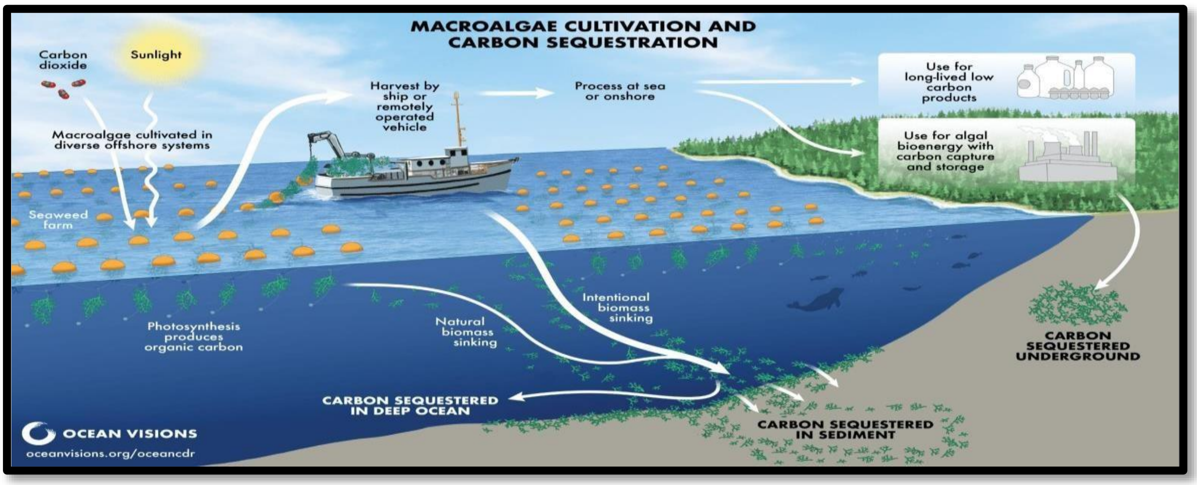


Figure II-4: Macroalgae cultivation and carbon sequestration pathways, including biomass harvesting, bioenergy conversion, and deep ocean storage.

II. 3. 2 Biochemical Composition of Algae:

➢ The compounds available in algal biomass include:

- ***Proteins:*** Spirulina platensis species consist of 60–70% protein by dry weight which positions them as one of the most protein-rich foods available **[34].**
- ***Carbohydrates:*** The dry weight of Chlorella vulgaris contains 12–17% carbohydrates **[35].**
- ***Lipids:*** The lipid content of Nannochloropsis oculata varies between 8% and 50% of its dry weight **[36].**

Carbohydrates: The dry weight of Chlorella vulgaris contains 12–17% carbohydrates **[35].**
Lipids: The lipid content of Nannochloropsis oculata varies between 8% and 50% of its dry weight **[36].**

II. 3. 3 Key amino acids derived from algae include:

The most abundant amino acids in algal biomass include:

- ✓ Glutamic acid
- ✓ Aspartic acid
- ✓ Glycine
- ✓ Alanine

These amino acids possess amine (-NH_2) and carboxylic acid (-COOH) groups that react with CO_2, making them effective agents in carbon capture processes **[81].**

II. 3. 4 Extraction and Optimization of Amino Acids from Algae:

Amino acids extracted from algal biomass need optimization in order to reach their full potential as CO_2 capture agents. The effectiveness of extraction methods varies based on different algal species along with their cell wall composition and the amino acid profiles required **[82].**

- **Common Extraction Techniques:**

Table II-1: Common Extraction Techniques table **[82], [83]**.

Method	Principle	Advantages	Limitations
Acid Hydrolysis	Breaks down proteins using HCl or H_2SO_4	High amino acid yield	Destruction of heat-sensitive amino acids
Enzymatic Hydrolysis	Protease enzymes break peptide bonds	Selective, mild conditions	Enzyme cost and slower rate
Microwave-Assisted Extraction (MAE)	Uses microwave energy to rupture cells	Time-saving, high efficiency	Requires moisture, may degrade amino acids
Ultrasound-Assisted Extraction (UAE)	Acoustic cavitation enhances solvent access	Energy-efficient, suitable for scale-up	May alter sensitive biomolecules

II. 4 Comparative Evaluation of Bio-Based CO_2 Capture Methods:

This section assesses the performance of algae-derived amino acids as CO_2 absorbents, compared with both conventional and emerging natural alternatives [47].

II. 4. 1 Ionic Liquids:

The distinctive properties of these salts enable them to dissolve CO_2 well in particular settings. The special qualities of ionic liquids (ILs) position them as beneficial natural substitutes for CO_2 capture because of their exceptional features. Let's examine the various ionic liquid types alongside their distinct benefits for CO2 capture applications **[47].**

II. 4. 2 Types of Ionic Liquids:

The selection of cations (positively charged ions) and anions (negatively charged ions) in an ionic liquid significantly influences its CO2 capture performance. Here are some key combinations:

• ***Ammonium-based ILs***: Known for their high CO2 solubility, ammonium based ILs can be tailored for specific applications through anion selection.
• ***Imidazolium-based ILs:*** These commonly used ILs possess good thermal stability and tuneable CO2 capture capacity depending on the anion selection.
• ***Phosphonium-based ILs:*** These ILs offer excellent chemical stability and tunability, making them suitable or harsh CO2 capture environments **[47].**

II. 4. 3 Biopolymers:

Which originate from natural sources, constitute an intriguing category of polymers characterized by a variety of applications and properties. In contrast to synthetic polymers, biopolymers are closely associated with the biological processes of living organisms and include proteins, carbohydrates, nucleic acids, and lipids. Their distinctive features, such as biodegradability and the ability to be sourced renewably, have generated considerable interest in both scientific research and industrial applications. This introduction provides an overview of the essential characteristics of biopolymers, paving the way for a deeper examination of their extensive applications and implications **[48].**

II. 4. 4 Types of Biopolymers:

Biopolymers include a diverse array of natural materials, such as:

- ***Chitosan:*** Sourced from chitin in crustacean shells, chitosan demonstrates significant potential for CO_2 capture due to its plentiful amine groups that readily interact with CO_2 **[48].**
- ***Cellulose:*** As the most prevalent organic polymer on the planet, cellulose can be chemically modified with various functional groups to improve its capacity for CO_2 capture **[49], [50].**

- ***Alginate:*** This polysaccharide, derived from brown algae, can form hydrogels with adjustable properties that are suitable for CO_2 capture **[51], [52].**

II. 4. 5 Algal Amino Acids vs. MEA-Based Solvents:

Table II-2: Comparative Evaluation of Algal Amino Acids and MEA-Based Solvents [7].

Criteria	Algal Amino Acids	MEA (Monoethanolamine)
Source	Renewable (e.g., Spirulina)	Petrochemical-derived
CO_2 Absorption Mechanism	Carbamate + Bicarbonate formation	Carbamate formation
Thermal Stability	High (>100 °C)	Moderate (Degrades ~80– 100 °C)
Degradation Products	Biodegradable	Toxic
Energy Requirement for Regeneration	Lower (~2.3–2.5 GJ/ton CO_2)	Higher (~3.0–3.5 GJ/ton CO_2)
Environmental Impact	Minimal (non-toxic)	Corrosive and environmentally harmful
Cost & Availability	Still under scale-up	Commercially established

- Remark: Algal amino acids present an environmentally friendly and thermally stable substitute for MEA while producing less environmental and energy impacts. On the other hand, large-scale implementation is still under development.

II. 4. 6 *Algae vs. Ionic Liquids:*

Table II-3: Comparative Evaluation of Algae and Ionic Liquids [47].

Criteria	Algae-Derived Amino Acids	Ionic Liquids (ILs)
Toxicity	Non-toxic	Moderate to high (depending on cation/anion)
Biodegradability	Excellent	Often poor
Viscosity	Low to moderate	High (limits mass transfer)
Synthesis Complexity	Low	High
Scalability	Feasible with biorefineries	Limited due to cost
CO_2 Capacity	Moderate to high (via amine groups)	Very high (up to 0.5 mol/mol)

- Remark: While ILs demonstrate high CO_2 solubility, their high cost, toxicity, and viscosity hinder large-scale applications. Algae-based systems are safer and more sustainable.

II. 4. 7 *Algae vs. Biopolymers :*

Table II-4: Comparative Evaluation of Algae and Biopolymers [48].

Criteria	Algal Amino Acids	Biopolymers (e.g., Chitosan, Cellulose)
Absorption Mechanism	Chemical reaction (carbamate/bicarbonate)	Physical adsorption and ion exchange
Surface Area	Variable	Typically high (porous structures)
CO_2 Selectivity	High	Moderate
Stability in Harsh Conditions	Good	Poor to moderate
Functionalization Flexibility	Moderate	High (can be modified for performance)
Renewability	Excellent	Excellent

Remark: Algal extracts contain reactive amines which demonstrate superior adsorption-based capture efficiency compared to biopolymers because of their high surface area and structural flexibility.

II. 5 Enzymes in Enhancing Algal-Based CO_2 Capture:

Enzymes provide a distinct method for capturing CO2 due to their biological selectivity and catalytic efficiency.

II. 5. 1 *Types of Enzymes:*

Carbonic anhydrases (CAs) are specific enzymes highly effective in capturing CO_2. These naturally occurring enzymes speed up the conversion of CO_2 into bicarbonate (HCO3-) and protons (H+).

Subtypes of Carbonic Anhydrases: Various subtypes of CAs exist, each with different activity levels and optimal conditions for operation. Some commonly studied subtypes include:

- **α-CAs:** These are the most prevalent and extensively researched CAs, recognized for their high catalytic efficiency.
- **β-CAs:** These enzymes tend to be more stable at elevated temperatures, making them potentially suitable for more demanding industrial settings.
- **γ-CAs:** These newly identified CAs possess unique characteristics, such as metal-independent activity, offering potential for further development **[38].**

Mechanism of Action: CAs act as catalysts by facilitating the following reaction:

$$\mathbf{CO2 + H2O \rightleftharpoons HCO_3^- + H^+}$$

This reaction efficiently transforms CO_2 from its gaseous state into a dissolved form (HCO3-), which can subsequently be captured or used in downstream processes **[38].**

II. 5. 2 *Advantages:*

- Reduced energy for regeneration.
- Better CO_2 capture at lower pH.
- Suitable for mild industrial conditions.

II. 5. 3 *Challenges:*

- High manufacturing cost and enzyme stability.
- Log-term operation or high temperature exposure increases the risk of denaturation.

II. 6 Industrial Implementation: Challenges and Future Prospects:

The section discusses real-world constraints alongside upcoming directions for expanding algae-based carbon dioxide capture systems including their technological and economic and environmental aspects.

II. 6. 1 *Scaling Up Algae-Based CO_2 Capture:*

➢ **Bioreactor Technology:**

- The use of large-scale algal cultivation needs photo-bioreactors alongside open ponds.
- Photo-bioreactors provide controlled growth conditions but represent high operational expenses.
- The combination with flue gas from industrial facilities maximizes the effectiveness of CO_2 delivery **[39].**

➢ **Harvesting and Extraction Challenges:**

- The combination of high water content together with small cell size of algae cells makes their energy-demanding harvesting process.
- The development of efficient dewatering procedures combined with amino acid extraction remains at an early stage **[40].**

➢ **Process Optimization:**

- The production output of the system depends on the specific levels of light intensity along with nutrient availability and CO_2 concentration.
- Scientists are currently investigating genetically modified strains which demonstrate potential for increased amino acid production **[41].**

II. 6. 2 *Future Innovations in Bio-Based CO_2 Capture:*

➢ Hybrid Systems:

- Combining algal amino acids with enzymes or nanoparticles for faster CO_2 absorption.

➢ Bio-electrochemical Integration:

- Integration with microbial fuel cells for simultaneous CO_2 capture and energy recovery **[42].**
  - ➢ Circular Bio-economy Approach:
- Integration with biofuel production and waste water for economic feasibility **[43].**
  - ➢ Environmental and Economic Impact of Bio-Based CO_2 Capture:
- Environmental Benefits:
- Zero emissions from regeneration process.
- Algae cultivation consumes CO_2 and releases O_2 [45].
  - ➢ Economic Assessment:
- Still cost-intensive at pilot scale, but co-production of bio-products (e.g., pigments, bio-fertilizers) can improve profitability.
- Potential to benefit from carbon credits in regulated markets **[46].**

II. 7 **Conclusion:**

This chapter has explored the potential of algae-derived amino acids as sustainable alternatives to conventional amine solvents in CO_2 capture technologies. By examining their natural occurrence, absorption chemistry, industrial applications, and the advantages they offer over traditional methods, it is evident that bio-based approaches especially those involving algae offer promising environmental and operational benefits.

Comparative evaluations with ionic liquids, biopolymers, and MEA-based solvents underscore their feasibility in industrial applications, despite some technical challenges. Additionally, innovations such as enzyme integration and hybrid systems, along with case studies on scaling and economic potential, highlight the growing relevance of this field.

Overall, algae-based amino acids stand out as an environmentally friendly and potentially efficient solution for future CO_2 capture strategies.

CHAPITRE III:

PRACTICAL WORK

Chapter III: Practical work

III. 1 Introduction:

Enzymes extracted from algae refer to those isolated and purified from algal sources, including two different types:

- **Macro algae:** called ''seaweeds'' large, multicellular, photosynthetic marine organisms that can grow from 50cm up to 60 metres in length.
- **Microalgae:** unicellular, photo synthetics organisms found in fresh water and marine environments, their size from 0.2µm to a few hundred micrometres and the most commonly are between 2 and 50µm, like examples: chlorella around 5µm, sripulina exceeding 50µm.

These enzymes act like a biological catalysts and can have unique proprieties compared to those from land plants, making them valuable from industries, pharmaceutical, and environment application, so Extracting enzymes from algae involves breaking down the tough algal cell-walls often using mechanical ,chemical or enzymatic methods to release the enzymes which are then purified for a special use.

This chapter is divided into two main sections:

1) The practical work (Chapter 1).
2) The discussion of the results obtained (Chapter 2).

Our primary main goal in this chapter s to define the different types of algae used in this study. Since algae contain proteins (enzymes), the focus is on how to extract these enzymes from our main source algae and subsequently purify and preserve them for use in our target application: the absorption of CO_2 emissions from industrial sources.

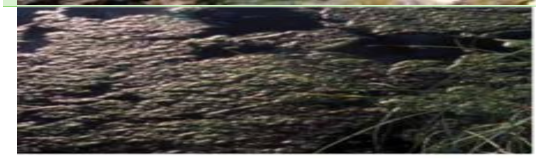
Our secondary goal is to find out which enzyme give us a good result and developed it well for its different applications latter. Finally the enzymes extracted represent our natural alternative solution for CO_2 emissions especially in the industry.

III. 2 Picking up algae:

In this study, a total of 12 algae samples were used and previously collected samples (coded A1 to A12). These algae were obtained from various freshwater and marine environments across different regions of Algeria.

The collected algae were coded for traceability and experimental comparison, as shown in the table below. Each sample was later subjected to microscopic identification and enzymatic extraction procedures in the laboratory.

Table III-1: Various algae collected

Photo	Figure name	Area
	A1	Touggourt
	A2	Ghardaia
	A3	Khenchla
	A4	Touggourt"Megarine"
	A5	Touggourt"Temacine"
	A6	Ouargla"Ain El Baida"
	A7	Ourgla"Sidi Khouiled"
	A8	Jijel"Boumarchi"

	A9	Jijel”Grand fere”
	A10	Annaba”Plage el safia”
	A11	Annaba”Hippon”
	A12	Tougourt ”Temacine”

III. 3 Identification of used algae:

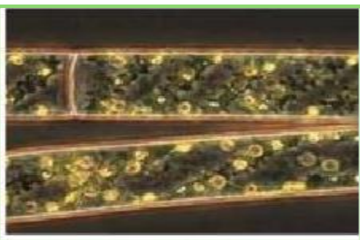
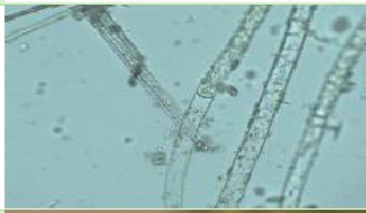
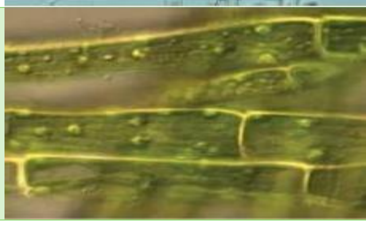
Algae are define by the observation under a microscope, the photos of our simples have token in the laboratory of Water and Environmental Engineering at university of Kasdi Merbah Ouargla.

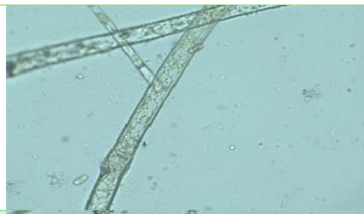
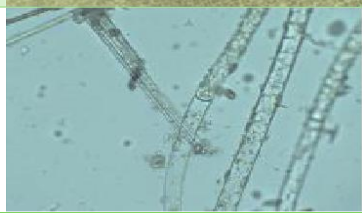
Figure III-1: simple’s preparation for microscope vision.

Figure III-2: the microscopic vision of our simples.

The collected algae have been defining by a name and a code described in this following table:

Table III-2: Name and symbol for algae

Code	Name of algae	Area	Class(family)	Photo
A1	***SPIROGYRA***	Tougourt	***Zygnematophyceae***	
A2	***NAVICULA***	Ghardaia	***Chlorophyceae “macroalgea”***	
A3	***OSCILA TORIA***	Khenchla	***Cyanohyceae***	
A4	***CLADOPHORA***	Tougourt ‘’Megarine’’	***Ulvophyceae***	
A5	***OEDOGONIUM***	Tougourt ‘’Temacine’’	***chlorophyceae***	

A6	***TRIBONEMA***	Ouargla ‘’Ain ElBeida’’	***Xanthophyceae***	
A7	***OEDPGONIUM***	Ouargla ‘’Sidi khouiled’’	***chlorohyceae***	
A8	***Cladophora***	Jijel ‘’Boumarchi’’	***Ulvophyceae***	
A9	***Ulvaceae***	Jijel “Grand fere”	***Ulvophyceae***	
A10	***Saragassaceae***	Annaba “plage El safia”	***phaeophyceae***	
A11	***Ulvaceae***	Annaba “Hippon”	***Ulvophyceae***	
A12	***Cladophora***	Tougourt “Temacine”	***Ulvophyceae***	

III. 4 **Definition of algae:**

- ✓ **.A1:***spirogyra* is a green algae found in freshwater environments such as pounds and streams, it is characterized by long and unbranched chains of cylindrical cells, each contain spiral shaped chloroplasts , that gives the name of genus of filamentous freshwater yellow green , from class of Zygnematophyceae**.[53-55].**

- ✓ **.A2:** *Navicula* is a genus of freshwater diatoms (type of algae) characterized by boat shaped elongate cell, with silicified cell wall called a “Frustule” composed of two overlapping halves. Navicula species typically have 2 chloroplasts per cell with rod shaped pyrenoids and are commonly found in ponds, reveres, and moist soils, from class of Chlorophyceae. **[56-58].**

- ✓ **.A3:** *Oscila Toria* is a genus of filamentous blue-green algae; we found it also in freshwater like pounds streams and ditches. Oscila toria gets its name from the show oscillating movement of its filamentous which help it glide throw water its reproduced mainly fragmentation, where sections of the filament and grow into new individuals. Oscila toria often forms blue-green mats on submerged surfaces and can sometimes cause and other issues in water supplies. [**59-66].**

- ✓ **.A4, A8, A12:** *Cladophora* is a genus of reticulated filamentous green algae in the class of Ulvophyceae. It forms branched filamentous structures composed of large cylindrical cells that create long regularly branched growths, often forming dense mats or tufts in aquatic environments. Cladophora grows attached to rocks or other subtracts in freshwater and marine habitats worldwide and can impact eco-systems by forming dance mats that effect light and oxygen levels. It reproduces bath sexually and asexually, with motile zoospores and biflagellate gametes**.[67-68].**

- ✓ **.A5, A7:** *Oedogonium* is a genus of filamentous unbranched green algae commonly found in freshwater environments such as pounds, lakes and ditches. The filamentous are typically one cell thick and maybe attached to other plants or free floating as masses each cell contains a large central vacuole and a netlike (reticulate) chloroplasts. Oedogonium reproduces both sexually and asexually with distinctive reproductive structure visible during contain life stages .it is easily recognized by its cylindrical cells and the presence of apical rings formed by successive cell division**.[69-76].**

- ✓ **.A6**: *Tribonema* is a genus of filamentous freshwater yellow-green algae class of Xanthophyceae its filaments are unbranched and made of cylindrical cells, each with a cell wall that consists over lapping H-shaped pieces. Tribonema contain many disc shaped chloroplasts that are green or golden in colour and lacks starch storing lipids instead. It is commonly found in pounds, ditches and streams and is notable for its potential in bio fuel production due to its high lipid content**.[77-79].**

All algae play an important role in aquatic eco system because they are the foundational primarily producers in the food web they perform photosynthesis converting water and carbon dioxide into organic matter (sugars) and releasing oxygen as a by-product which support aquatic life such as fish and invertebrates.

III. 5 Simples preparation:

After the manual collection of our algae from different areas that are mentioned in the previous table. We have done a carful rinsing to eliminate salinity and impurities contained in the algae, then a controlled drying for minimum 2 days, with air to preserve the enzymatic activity while facilitating the manipulation on it. Finally the step of grinding and homogenization of our main simples, we grind the dry algae's into fine powder reduction to increase the extraction surface and facilitate access to intracellular enzymes.

- The two steps of preparation o our simples are defining in this coming table :

Table III-3: steps of preparation

Step of controlled dry (with air) of simples.	**Simple's preparation.**
 **Controlled dry for simples A6 and A7.**	 Simples A6 and A7.  Simples from A1 to A5.

III. 6 Definition of enzyme extracted from algae:

In this study we are going to focus on one enzyme extracted from our main source ”algae” it called “Cas” carbonic anhydrise enzyme.

III. 6. 1 Definition:

The Cas is a zinc containing enzyme that catalyzes the reversible hydration of carbon dioxide (CO_2) to carbonic acid (H_2Co_3), which rapidly dissociates into bicarbonate ($HCO^-{}_3$) and protons (H^+) this reaction is critical for maintaining acid base balance , (CO_2.) transport and PH regulation in biological systems.

- **function:** accelerate (CO_2) conversion to bicarbonate.
- **structure**: contain a zinc ion at its active site coordinated by histidine residues enabling rapid catalysis (up to 1 million reactions per second).
- **classes**: evolved independently into multiple forms (**α,** β, γ, δ, ε η, θ, λ).

The most known ones are:

α-Cas : highly active and well-studied carbonic enhydrase enzymes.

β-Cas : found especially in algae stable at higher temperature.

γ-Cas : unique, metallique Independent activity.

- **roles in algal sources:** the “Cas” enzymes are essential for photosynthetic carbon concentrating mechanics (CCMs) and environmental (CO_2) sensing.

III. 6. 2 Example:

CAH1/CAH3: found in green algae called chlamidomonas-reinharditii.DCA1/DCA2: found in dunaleilla salina it functions in hyper saline conditions to enhance CO_2 uptake.

As a summery those enzymes play a key role in CO_2 capture by speeding up its conversion into bicarbonate ($HCO^-{}_3$).

III. 7 The extraction of the "Cas" enzymes from algae:

This operation is done with 2 different protocols:

III. 7. 1 Protocol 1:

We needed in this protocol those following materials and tools presented in this table:

Table III-4: materials and tools Protocol 1 **[84-85]**

Materials	Tools
Distilled water **NaOH powder** **Algae powder** **Ethanol**	Beaker spatula Centrifigyuse dryer Earline Mayer filtration papers Electric balance, funnel, bath water.

III. 7. 2 Protocol steps:

The protocol was implicated by following these steps: [**84-85]**

- ✓ In dry and clean beaker we are going to put m=2.5g of algae powder with v=250ml of distilled water, we obtained "sollution1". At the same time a saline solution was prepared by putting m=8g of NaOH powder in v=200ml of distilled water.
- ✓ We added the saline solution in sollution1 and mix the mixture well.
- ✓ After that we have putted the mixture in a bath water fixed at T=37◦C with agitation for 30min, then we waited until it became at laboratory temperature, we obtained "sollution2".
- ✓ The next step is adding in "sollution2" a sufficient amount of solvent "Ethanol" **v=40ml,** we obtained mixture2, this latter was placed in specific tubes directly to the centrifugyuse in a run of 5000rpm for 20min. After the centrifugation we obtained sediment left at the bottom of tubes, this sediment represent a protein contained our required enzyme "Cas".
- ✓ Finally the step of filtration using filter papers and drying with air for 2 days , then their storation in a specific tubes to use it latter.
- ✓ Note: in this protocol we have doubled the quantities of tools used five times to obtain much mass of enzymes finally.

III. 7. 3 Results:

Protein contained CA enzyme and other compositions. (96% enzyme and 4%: impurities).

Figure III-3: represents the application of protocol 1.

III. 7. 4 Protocol 2:

The tools and materiel that we needed them are presented in the table bellow:

Table III-5: the tools and materiel of Protocol 2 **[86].**

Materials	Tools
KH_2PO_4.	Beakers.
K_2HPO_4.	Earline mayors.
Distilled water.	Spatula.
Algae powder.	Electric balance.
NaoH powder.	Dryer.
	Filtration papers.
	Refrigerator funnels.
	Test tubes.
	Centrifigyuse.

III. 7. 5 Protocol steps:

We have followed these following steps to apply this protocol: **[86]**

- ✓ preparation of a mother solution which contain a mix of m'=2.98g (K_2HPO_4) with m''=0.2g (KH_2PO_4.) in V=400ml of distilled water and then we mix the mixture very well for 3min.
- ✓ A saline solution was prepared by putting m=2.8g of NaOH powder in V=400ml of distilled water at the same time.
- ✓ We have weighted m=1g of algae powder and added it in the mother solution with V=80ml of saline solution and of course we mix well.
- ✓ Preparation of testes tubes felt with the final mixture and put them in the centrifigyuse at run of 4500rpm for 20min.

- ✓ We obtained after the centrifigyuse a residue at the bottom of test tubes, that why we have displaced them carefully after they calm down completely whiteout mixing them.
- ✓ the final steps is to filter the residue obtained and waited until its dry completely after that we preserved the dry residue in a special tubes to use them latter.

III. 7. 6 Results:

The final extract obtained through this protocol consists primarily of proteins containing the targeted Carbonic Anhydrase (CA) enzyme, along with other minor biological components and impurities. The sediment collected after centrifugation represents the active enzyme fraction, which was successfully isolated from the algal biomass.

Figure III-4: *represent the application of the second protocol.*

Note: in this protocol we have doubled the quantities four times to get enough mass of the extracted enzyme.

Exemple:

We need m=0.745g of (K_2HPO_4) , so the quantity used heir m=2.98g.

III. 8 **Carbon dioxide experiment:**

This experiment was done to test the effectiveness of our enzymes extracted with both protocols 1 and 2.

- We have done heir an experiment of fermentation which represents the source of carbon dioxide as follows:

First of all we have been prepared 2 different solutions, solution 1 contained m=0.125g of enzyme powder obtained from one of the protocols and m=0.7g of salt in v=100ml of distilled water and the solution 2 is the fermentation one m=1.6g of yeast with m=4g sugar in v=100ml of distilled water, after that we link between them and create an isolated system verifying like this the coming figure:

Figure III-5: the operation of fermentation.

III. 8. 1 Results:

After the operation of fermentation the value of ph change in the two protocols, we have protocol 1 decreases and the second protocol increases.

III. 9 The measure of the conversion value of carbon dioxide via H+ ions using a multi-parameter device:

III. 9. 1 Definition:

A multi-parameter device is an instrument designed to messure several parameters simultaneously, often related to chemical physical or biological characteristics.

For example:

In water quality monitoring, a multi-parameter (sonde) can measure temperature, ph, dissolved oxygen, turbidity, conductivity and many other indicators in one compact instrument providing real time data for assessing water health.

In medical settings, a sonde monitor tracks vital signs like heart rate, blood pressure, oxygen saturation, respiratory rate, and ECG simultaneously to give a comprehensive view of a patient's condition.

In summery a multi-parameter device integrates multiple sensing capabilities into a single unit to efficiently monitor several parameters at once, improving data collection and analysis in fields like healthcare and environmental monitoring.

Figure III-6: the multi-parameter.

III. 10 conclusion:

Now we can say that we have been successfully extracting our enzymes especially “Cas” enzyme from an algal source from different area’s in Algeria, verifying this way our main goal.

The final step is to test the effectiveness of our enzymes extracted and this is coming and mentioned in the previous chapter (chapter2).

All this to achieve the basic goal of their extraction which is absorb the CO_2 emissions especially from industries.

CHAPTER IV:

RESULTS AND DESCRIPTION STUDY

Chapter IV: Results and description

IV. 1 Introduction:

After the practical work which ended by extracting our carbonic en-hydrase enzyme from our collected algae we obtained the coming results (mentioned in 2).

IV. 2 Results of test extraction:

The Test as a summery is to see the value of PH before and after the CO_2 absorption experiment for the different solutions. In this way we are going to see the effectiveness of our extracted enzymes.

Table IV-1: Change of the PH value before and after the CO_2 absorption experiment (protocol 1).

Enzymes extracted from:	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Ph before	8.62	8.61	8.63	8.6	8.46	8.63	8.1	8.25	9.16	8.6	8.4	8
Ph after	6.42	6.29	6.98	5.75	6.49	7.20	6.37	6.45	6.4	6.2	6.1	6
Yield %	80	88	84	92	96	84	84	88	92	80	96	80

To calculate the yield we have been apply this rule 1:

$$Y = (m2 \div m1) \times 100$$

With:

Y: *represent the yeild* (%).

M1: represent the initial mass m=2.5g.

M2: represent the final mass (according to the algae used).

the results of table XII.1 actively demonstrate persuasively that the best yield is for two algae's which are A5 and A11 (96%) while the lowest yield is for those tree A1, A10 and A12 (80%), So we can say that both A5 and A11 have the highest value of absorption unlike the A1,A10 and A12 which recorded the opposite thing.

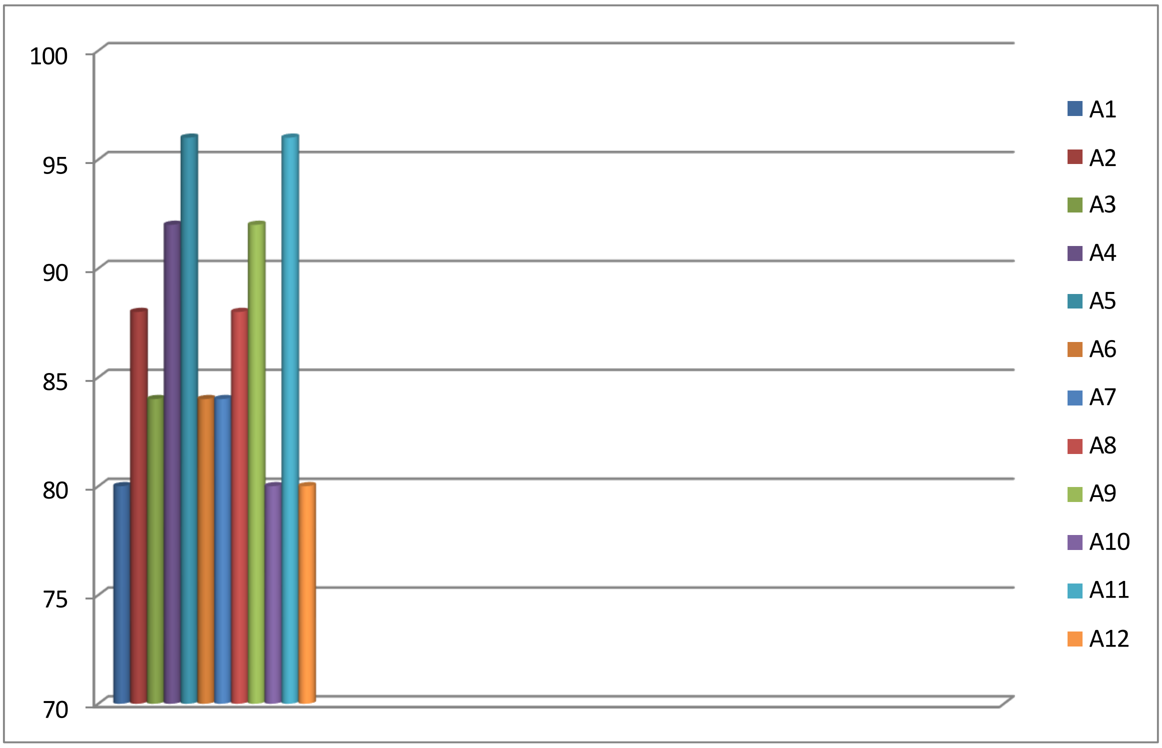


Figure IV-1: *graph bar sows the different yields for the algae used in protocol 1 from A1 to A12.*

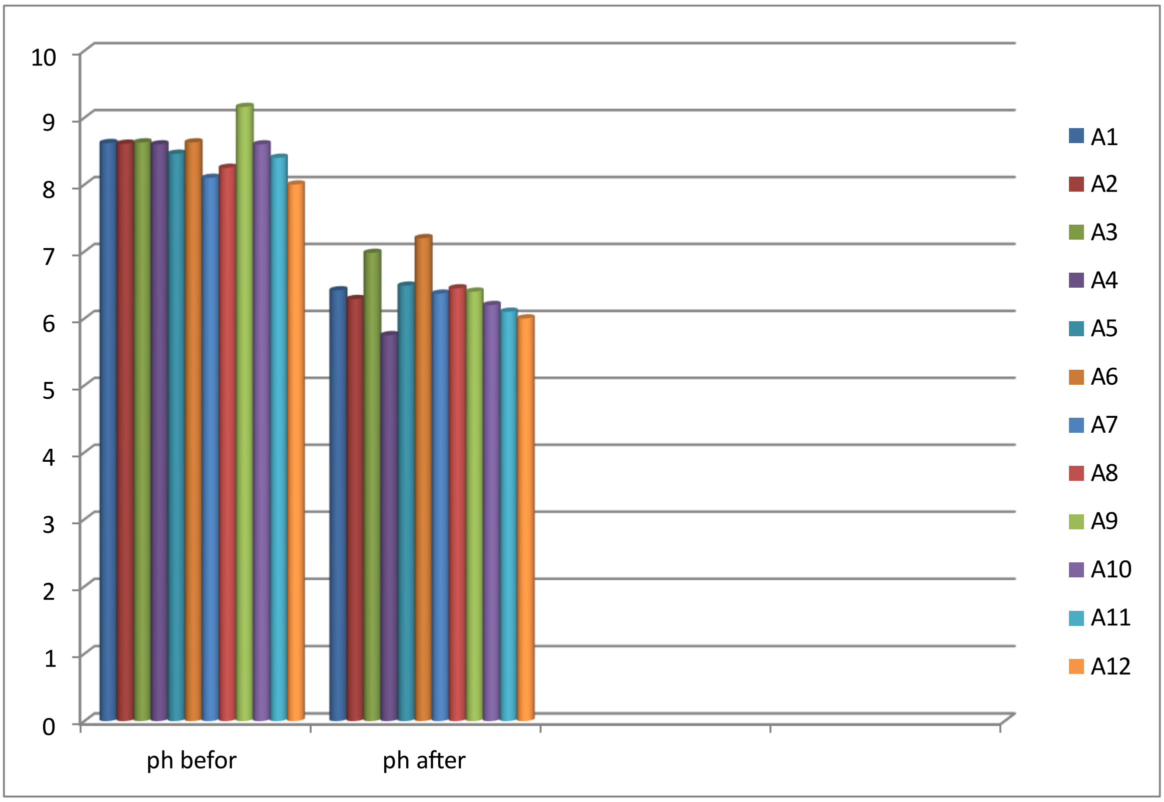


Figure IV-2: graph bar represent the change of PH before and after the absorption of carbon dioxide (protocol 1).

Table IV-2: represent the change of PH value before and after absorption of CO_2 experiment (protocol 2).

Enzymes extract from:	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
Ph before	11.23	11.20	11.21	11.20	11.10	10.9	10.65	10.85	10.81	10.9	10.2	10.1
Ph after	11.39	11.25	11.30	11.29	11.24	11.1	10.73	10.9	10.95	10.98	10.32	10.12
Yield (%)	30	50	20	40	20	10	13	70	60	45	60	30

The calculation of the yield is with the same precedent rule1 mentioned previously.

IV. 3 Comparison between protocol 1 and 2:

- ✓ First of all we have used the same algae in the both protocols with different experiment.
- ✓ Now to see which protocol has the best average yields we have
  2.5g (protocol 1) = 87% it gives for 1g (protocol 1) = 34.8% also we have
  1g (protocol 2) = 37%.

As we see the protocol 2 has the better average yield with (37%) so the enzymes obtained using the protocol 2 are more effective than protocol 1.

Based on the results obtained in the table XII.3 we have found that the best yields is for the enzyme extracted from the algae A8 with (70%), while the lowest yields is for A7 with only (13%) this means that in this protocol the best value or we can say the best absorber of carbonic dioxide is A8 and the less value is for A7.

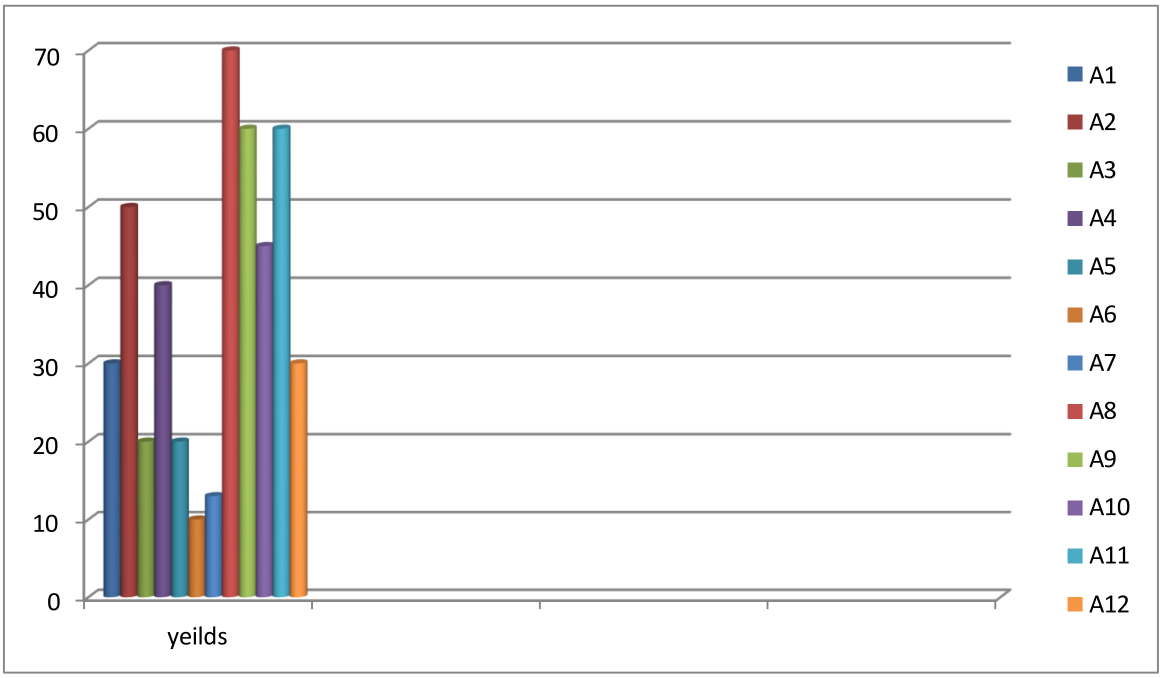


Figure IV-3: show us the different yields of our enzymes extracted (protocol 2).

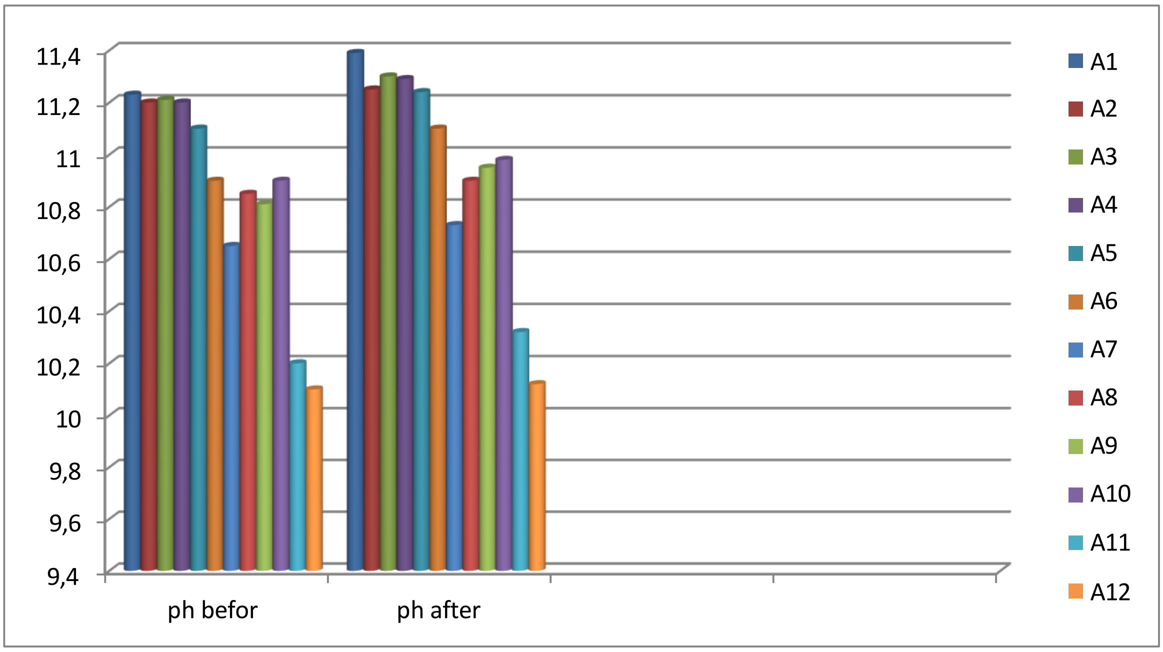


Figure IV-4: graph bar shows the PH value of enzymes before and after the absorption of carbon dioxide experiment (protocol 2).

IV. 4 Conclusion:

Now to sum up all this we have traditional and new techniques to absorb the CO_2 emission generally, the traditional one's are used in the industries but they use high energy which costs very expensive first of all and then they are not eco-friendly so we should replace them with some new techniques for example in our study we have been extract enzymes called carbonic unhydrase enzymes "Cas" from an natural source which is the algae to absorb the CO_2 emissions in an effective way , and finally as we all know the main goal of industries in their equipment is "less expensive and more effective" that's how we've got the target and the study ended successfully

GENERAL CONCLUSION

General conclusion

This thesis has shown that enzymes and amino acids derived from algae can be efficient and environmentally friendly substitutes for conventional amine capture systems for CO_2. Theoretical studies described the shortcomings of incumbent systems, including high energy required for regeneration and environmental risks. However, bio-based systems have less toxicity, biodegradation ability, and compatibility with renewable carbon prevention strategies **[1] [4].**

The laboratory experimentation, performed on various Algerian regions' diverse macro- and microalgae samples, verified the possibility of producing enzymes under laboratory conditions. The two extraction protocols' comparison showed that Protocol 2 yielded higher enzyme production and improved CO_2 capture capacity particularly for algae species such as Cladophora and Ulvaceae **[10].**

The findings confirm the viability of using bio-based technologies in industrial-scale carbon capture systems. Future research should prioritize optimizing the cultivation of algae, extraction processes for algal biomass, and hybrid bio-reactor design for operation in industrial flue gas conditions. Another aspect can be the integration of algal carbon capture with biofuel production and circular bio-economy systems for further economic feasibility **[11].**

In conclusion, nature-inspired solutions not only guarantee an environmentally friendly future but also offer pragmatic solutions to costly and polluting fossil-fuel technologies. The research advances the transition towards bioengineering and biorefineries as key pillars in environmentally aware industrial innovation. .

BIBLIOGRAPHICAL REFERENCES

1. Boulder, RMI Releases Pioneering Insights on Emissions Footprint of Global Oil Refining and Petrochemical Industries, CO – September 21.

2. C.C BY 4.0, Emissions from Oil and Gas Operations in Net Zero Transitions, May 2023.

3. Alan Krupnick, Joshua Linn, Richard D. Morgenstern, and Dallas Burtraw, Federal Climate Policy 105: The Industrial Sector, March 31, 2021.

4. C.C Article from: Making an Impact with Oil Refineries and CCUS Technology, 25 May 2021.

5. Ridha Ben Said and Ridha Ben Said,Department of Chemistry, College of Science and Arts, Qassim University, Ar Rass 51941, Saudi Arabia, ARTICLE October 1, 2020 A Unified Approach to CO_2–Amine Reaction Mechanisms.

6. Amine gas treating, Wikipedia, by "Wikipedia contributors." April 2025

7. Howard D. Goodfellow & Yi Wang (2nd ed., 2021) Book title: Industrial Ventilation Design Guidebook (Second Edition) Chapter/Section title: "Acid Gas Removal" Publisher: Elsevier Science p. 123–125, 2021.

8. Wenhao Jiang, Yuchen Lin, Comparative Review for Enhancing CO2 Capture Efficiency with Mixed Amine Systems and Catalysts, 2024 Sep 29.

9. Loachamin, D., Casierra, J., Calva, V., Palma‑Cando, A., Ávila, E. E., & Ricaurte, M. (2024, December 13). Amine‑Based Solvents and Additives to Improve the CO_2 Capture Processes: A Review. ChemEngineering, 8(6), Article 129.

10. Jiang, W., Lin, Y., Sun, C., Sun, Y., & Zhu, Y. (2024, September 29). Comparative Review for Enhancing CO_2 Capture Efficiency with Mixed Amine Systems and Catalysts. Molecules, 29(19), Article 4618.

11. Vega, F., Sanna, A., Navarrete, B., Maroto‑Valer, M. M., & Cortés, V. J. (2014, July 10). Degradation of amine‑based solvents in CO_2 capture process by chemical absorption. Greenhouse Gases: Science and Technology, 4(6), 707–733.

12. [Heat recovery solves carbon capture issues, Mar-2021.]

13. mith, J., & Lee, K. (2024). Design Considerations for Postcombustion CO_2 Capture With Membranes. In Main Technologies in CO_2 Capture (2.2.2 Membrane, Chapter 14). TechPress. pp. 123–140.

14. Membrane Gas Separation." Wikipedia, Wikimedia Foundation, 6 Feb. 2024, https://en.wikipedia.org/wiki/Membrane_gas_separation. Accessed 26 Apr. 2024.

15. IIF‑IIR. "Cryogenic Carbon Capture Technologies." International Institute of Refrigeration – News, April 29, 2022.

16. Wanison, Ramnarong, Wahyu Nurkholis Hadi Syahputra, Niti Kammuang lue, Pradit Terdtoon, and Phrut Sakulchangsatjatai. “A Review of Cryogenic Carbon Capture Research.” Thermal Science and Engineering Progress 61 (May 2025): Article 103562.

17. Wikipedia contributors. “Bioenergy with Carbon Capture and Storage.” Wikipedia, last edited February 13, 2025, 21:20 UTC. https://en.wikipedia.org/wiki/Bioenergy_with_carbon_capture_and_storage.

18. Hong, Wan Yun. 2022. “A Techno‑economic Review on Carbon Capture, Utilisation and Storage Systems for Achieving a Net‑Zero CO_2 Emissions Future.” Carbon Capture Science & Technology 3 (June): Article 100044.

19. Barlow, Hugh, Shahrzad S. M. Shahi, and David T. Kearns. 2025. Technology Readiness and Costs of CCS. Global CCS Institute, January 30.

20. Fernandes De Souza, Marcella, Erik Meers, and Silvio Mangini. “The Potential of Microalgae for Carbon Capture and Sequestration.” EFB BIOECONOMY JOURNAL 4 (November 4, 2024): Article 100067.

21. Title: Hollow Fiber Membrane Contactor for CO_2 Capture: A Review of Recent Progress on Membrane Materials, Operational Challenges, Scale-Up, and Economics

Link: ScienceDirect, 2024 Operational Challenges: Discusses issues like membrane wetting, material degradation, and scale-up difficulties. Economic Considerations: Addresses the cost implications of membrane fabrication and operation.

23. South Pole. “Carbon Removals with Biochar.” South Pole Blog, 2022,

24. Aneesh, A. M., and Ashish Alex Sam. 2023. “A Mini‑Review on Cryogenic Carbon Capture Technology by Desublimation: Theoretical and Modeling Aspects.” Frontiers in Energy Research 11 (May 23): Article 1167099.

25. Prasad, Ravindra, Sanjay K. Gupta, Nisha Shabnam, Carlos Yure B. Oliveira, Arvind Kumar Nema, Faiz Ahmad Ansari, and Faizal Bux. 2021. “Role of Microalgae in Global CO_2 Sequestration: Physiological Mechanism, Recent Development, Challenges, and Future Prospective.” Sustainability 13 (23): Article 13061.

26. Rath, Gourav Kumar, Gaurav Pandey, Sakshi Singh, Nadezhda Molokitina, Asheesh Kumar, Sanket Joshi, and Geetanjali Chauhan. 2023. “Carbon Dioxide Separation Technologies: Applicable to Net Zero.” Energies 16, no. 10 (May 15): Article 4100.

27. Ashour, Mohamed, Abdallah Tageldein Mansour, Yousef A. Alkhamis, and Mostafa Elshobary. 2024. “Usage of Chlorella and Diverse Microalgae for CO_2 Capture – Towards a Bioenergy Revolution.” Frontiers in Bioengineering and Biotechnology 12 (August): Article 1387519.

28. https://pubs.rsc.org/en/content/articlelanding/2021/ta/d0ta10583 3, 2021

29. Cysbio. 2023. “Disruptive Platform for Sustainable and Low‑Cost Amino Acid Production (BioBAA).” Horizon 2020 Grant No. 101009882, December 1, 2020–May 31, 2023.

30. Wang, Yufeng, Song Zheng, Luyao Xia, Elisa Roleda Yu, Wei Zhang, and Xinjuan Ma. 2021. “Amino Acids in Microalgae: Diversity, Biosynthesis, and Applications.” Algal Research 56 (2021): Article 102286.

31. Barzagli, F., et al. (2023). Eco-friendly CO_2 capture using amino acid-based solvents: Technological insights and challenges. Chemical Engineering Journal, 451, 139025. https://doi.org/10.1016/j.cej.2022.139025

32. Khan, M.I., et al. (2022). Microalgae as a source of bioactive compounds for carbon capture and biorefinery. Journal of Environmental Chemical Engineering, 10(2), 107347. Published: 9 June 2022.

33. Sang Sefidi, Vida, and Patricia Luis. “Advanced Amino Acid‑Based Technologies for CO_2 Capture: A Review.” Industrial & Engineering Chemistry Research, vol. 58, no. 24, 18 Dec. 2020, pp. 11637–11657.

34. Karkos, P. D., et al. (2023). Bioactive Compounds from Spirulina spp.—Nutritional Value, Functional Properties, and Applications. MDPI Foods, 11(9), 257.

35. Elsevier. (n.d.). Chlorella. In ScienceDirect Topics. Retrieved [april 2], from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chlorella

36. Silva, A. F., et al. (2022). Production of Antioxidants and High Value Biomass from Nannochloropsis oculata. MDPI Marine Drugs, 20(9), 552

37. memoire fin d'étude Master 2 raffinage p ;28 , 2024 Contribution to the study of natural alternatives for activator to Amino Acids for the CO2 removal in refining.

38. memoire fin d'étude Master 2 raffinage p ;18 , 2024 Contribution to the study of natural alternatives for activator to Amino Acids for the CO_2 removal in refining.

39. Iglina, Tatyana, Pavel Iglin, and Dmitry Pashchenko. 2022. “Industrial CO_2 Capture by Algae: A Review and Recent Advances.” Sustainability 14, no. 7 (March 23): Article 3801.

40. Richardson, James W., et al. “Harvesting and Extraction Technology Contributions to Algae Biofuels Economic Viability.” Algal Research, vol. 5, no. 1, July 2014, pp. 70–78.

41. Li, Gang, Wenbo Xiao, Tenglun Yang, and Tao Lyu. 2023. “Optimization and Process Effect for Microalgae Carbon Dioxide Fixation Technology Applications Based on Carbon Capture: A Comprehensive Review.” C – Journal of Carbon Research 9, no. 1 (March 16): Article 35.

42. Article: "Industrial Wastewater Treatment with Simultaneous Energy Recovery Using Microbial Fuel Cells – A Review" Authors: Brunschweiger, S., Hofmann, T., & Glas, K. Journal: Bio Resources Year: 2021.

43. Pal, Priti, Akhilesh Kumar Singh, Rajesh Kumar Srivastava, Saurabh Singh Rathore, Uttam Kumar Sahoo, Sanjukta Subudhi, Prakash Kumar Sarangi, and Piotr Prus. 2024. “Circular Bioeconomy in Action: Transforming Food Wastes into Renewable Food Resources.” Foods 13, no. 18 (September 23): Article 3007.

44. Article: "Circular Bioeconomy in Action: Transforming Food Wastes into Renewable Food Resources" Authors: Pal, P., Singh, A. K., Srivastava, R. K., Rathore, S. S., Sahoo, U. K., Subudhi, S., Sarangi, P. K., & Prus, P.Journal: Foods Year: 2024.

45. Kumar, A., D. Sharma, A. Tiwari, and G. Dixit. 2022. “Industrial CO_2 Capture by Algae: A Review and Recent Advances.” Sustainability 14, no. 7: 3801.

46. García, J. L., I. de Godos, and R. Muñoz. 2021. “Techno-Economic Analysis of Microalgae Related Processes for CO_2 Capture and Utilization.” Algal Research 56: 102288.

47. memoire fin d'étude Master 2 raffinage p;17 , 2024 Contribution to the study of natural alternatives for activator to Amino Acids for the CO2 removal in refining.

48. Aghaie, M., Rezaei, N., & Zendehboudi, S. (2018). "A systematic review on CO_2 capture with ionic liquids: Current status and future prospects." Renewable and Sustainable Energy Reviews, 96, 502–525. (Specifically mentions amine-functionalized materials like chitosan for CO_2 capture) Chen, X., Yang, H., Yan, N., & Chen, Y. (2017). "Application of chitosan and its derivatives in CO_2 capture." Carbohydrate Polymers, 173, 149–157.]

49. Foston, Marcus, and Arthur J. Ragauskas. 2010. “Changes in Lignocellulosic Supramolecular and Ultrastructure during Pretreatment for Bioenergy Production.” Biofuels, Bioproducts and Biorefining 4 (2): 134–145.

50. Huang, Y., Liu, M., and Yin, X. 2021. “Functionalized Cellulose-Based Materials for CO_2 Capture: A Review.” Journal of Cleaner Production 285: 124861.

51. Abubakar, L., and N. Sulaiman. 2020. “Recent Progress in Alginate-Based Materials for CO_2 Capture: A Review.” International Journal of Biological Macromolecules 165: 1–17.

52. Abubakar, L., and N. Sulaiman. “Recent Progress in Alginate-Based Materials for CO_2 Capture: A Review.” International Journal of Biological Macromolecules, vol. 165, 2020, pp. 1–17.

53. BNO Team. 2024. “Spirogyra.” Biology Notes Online, April 9, 2024. Accessed April 29, 2024.

54. Sapkota, Anupama. 2022. “Spirogyra.” Edited by Sagar Aryal. Microbe Notes, March 8, 2022. Accessed April 4, 2025.

55. Lewin, Ralph A., Robert A. Anderson, and others. 2025. “*Spirogyra*.” Updated by Adam Augustyn. Encyclopaedia Britannica, April 21, 2025. Accessed May 1, 2025.

56. Pertuzzella, Melissa. 2025. “Navicula.” Encyclopaedia Britannica, March 22, 2025. Accessed May 2, 2025.

57. North Carolina Division of Water Resources. 2017. Navicula. North Carolina Department of Environmental Quality. Accessed May 1, 2025..

58. Wangmo, Tashi, and Tenzin Yangkey. 2022. [Document Title]. Department of Environmental and Life Sciences, Sherubtse College, Royal University of Bhutan. Accessed May 5, 2025.

59. Taylor, Christopher. 2020. “Navicula.” Catalogue of Organisms, December 22, 2020. Accessed May 5, 2025.

60. Rogers, Kara. 2025. “Oscillatoria.” Encyclopaedia Britannica. April 29, 2025. Accessed May 5, 2025.

61. Sahoo, Animesh. 2022. “[Article Title].” Biology Learner, November 12, 2022. Accessed May 5, 2025.

62. BYJU’S. 2023. “Oscillatoria – Structure and Reproduction.” April 20, 2023. Accessed May 5, 2025.

63. Sahoo, Animesh. 2022. “Oscillatoria – Salient Features, Occurrence, Thallus Structure, Reproduction.” Biology Learner, November 12, 2022. Accessed May 5, 2025.

64. Hyashi, Kendra, Jenny Q., and others. 2022. “[Title of article].” Ocean Data Center, UC Santa Cruz, January 17, 2022. Accessed May 6, 2025.

65. North Carolina Division of Water Resources. [Title of article]. North Carolina Department of Environmental Quality. Accessed May 2025.

66. Aquasabi (germen academic site) written by Professors and studiers on the morphology of genus cladophora, Marsin and J.Tomas on March, 20, 2019. And took on May, 06, 2025.

67. Britannica.com article written by Mellissa petrozzello a researcher in plants and renewable energy and environmental engineering on April, 2025 and took on May, 2025.

68. Journal of Hazardous Materials written by Molly.c on April, 2022 and took on May, 2025.

69. Wikipedia contributors. "Oedogonium." Wikipedia. Last modified August 2024. Accessed May 2025.

70. "Oedogonium." Encyclopædia Britannica. Accessed May 2025.

71. Oxford University Press. "Oedogonium." Oxford Reference. Accessed May 2025. https://www.oxfordreference.com/display/10.1093/oi/authority.20110803100246103

72. Oedogonium link (scientific site) article written on May, 2020 and took on May, 2025. https://www.britannica.com/science/Oedogonium

73. Oedogonium link (scientific site) article written on May, 2020 and took on May, 2025. https://fmp.conncoll.edu/silicasecchidisk/LucidKeys3.5/Keys_v3.5/Carolina35_Key/Media/H

74. John, David M., B. A. Whitton, and A. J. Brook, eds. The Freshwater Algae of North America: Ecology and Classification. San Diego: Academic Press May, 2025. https://en.m.wikipedia.org/wiki/Oedogonium_

75. Guiry, M. D., and G. M. Guiry. "Oedogonium." Inanimate Life. LibreTexts. 2019.

76. [From Librettists biology (it's an online library) took on May, 2025.] https://fmp.conncoll.edu/silicasecchidisk/LucidKeys3.5/Keys_v3.5/Carolina35_Key/Media/Html/Oedogonium_Main.html

77. Oedogonium – Main Page. Lucid Keys / Carolina35 Key. Accessed May 2025.
(same details as ref 72).

78. "Microalgae." ScienceDirect Topics. Elsevier. Accessed may 1, 2025.**79.** Microalgae: A Promising Source of Valuable Bioproducts - PMC .

80. Sarwer A, Hamed SM, Osman AI et al. Algal biomass valorization for biofuel production and carbon sequestration: a review. Environmental Chemistry Letters. October 2022;20:2797.

81. Mata et al. (2023), "The future of algal proteins: Innovations in extraction and functionality", Trends in Food Science & Technology, Vol 134, p 75–88.

82. Nitsos, Current and novel approaches to downstream processing of microalgae: A review, Biotechnology Advances, (2020).

83. Herrero, Macroalgal Proteins: A Review, Journal of Applied Phycology (2022).

84. The previous thesis titeled by Contribution to the study of natural alternatives for activator to amino acids for the CO_2 removal in refining this latter presented by both of Hamlaoui Amor and Chine Nadjib on 10/06/2024.

85. Acadimic web site called Frontiers.

86. NIH: national library of article medicine extracted from the national center for biotechnology information.