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Preliminary Considerations on the Co-Production of Biomethane and Ammonia from Algae and Bacteria

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Abstract

Ammonia is a critical compound for numerous industrial processes; however, the conventional methods for its production present substantial environmental challenges. Co-producing biofuels and ammonia from biomass through anaerobic digestion offers a promising alternative to address these concerns. This study presents a theoretical assessment of the co-production of biomethane and ammonia from microalgae and cyanobacteria, utilising water from abandoned mine and quarry pit-lakes—specifically focusing on the Alessandria district as a case study. The analysis is based on the average values reported in the literature for the anaerobic digestion of selected biomass types. The results highlight *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Chlorella* spp., and *Chlorella pyrenoidosa* as the most promising species due to their superior yields of both ammonia and biomethane. This work aims to promote new opportunities for repurposing disused mining pit-lakes, contributing to the development of sustainable pathways for the integrated production of biofuels and ammonia. In this context, exploring integrated biorefinery systems within a bio-based economy represents an auspicious direction for future research, potentially enhancing the process efficiency and reducing costs.

Keywords: ammonia; absorption cooling systems; biofuels; natural fluids; non-equilibrium thermodynamics

Academic Editors: Said Al-Hallaj and Alessandro Dell'Era

Received: 27 March 2025
Revised: 13 June 2025
Accepted: 24 June 2025
Published: 26 June 2025

Citation: Lucia, U.; Grisolia, G. Preliminary Considerations on the Co-Production of Biomethane and Ammonia from Algae and Bacteria. *Inventions* **2025**, *10*, 47. https://doi.org/10.3390/inventions10040047

1. Introduction

1.1. Environmental Challenges and the Need for New Fuels

Increasing concerns about climate change have intensified the pursuit of renewable energy sources and more sustainable industrial practices. In this context, the use of non-edible biomass to produce non-fossil fuels, coupled with other valuable co-products, is being actively explored [1–3]. Among the promising candidates, microalgae and cyanobacteria have garnered significant attention due to their fast growth rates, high photosynthetic efficiency, and ability to accumulate substantial amounts of lipids, carbohydrates, proteins, and other valuable molecules, making them a suitable feedstock for biofuel production [4,5]. Moreover, these microorganisms can be cultivated using unconventional water sources such as wastewater [6,7], mining effluent [8], and mine water in post-mining landscapes [9,10]. These waste streams are often rich in nutrients like nitrogen and phosphorus, making them suitable media for algal growth, reducing the cost for nutrients [11,12] while simultaneously addressing environmental concerns. For instance, *Chlorella vulgaris* has demonstrated efficacy in removing inorganic nutrients and heavy metals from various wastewater types, including municipal and industrial effluent [13]. Similarly, *Scenedesmus* species have been successfully cultivated in treated acid mine drainage, contributing to both biomass production and water remediation [14]. These approaches not only mitigate the environmental impact of wastewaters but also reduce the costs associated with freshwater and nutrient inputs in algal cultivation. Furthermore, this dual functionality underscores a virtuous approach: treating water that needs bioremediation while producing valuable bioresources.

Among biofuels, biogas has been recognised as a promising one, with significant potential to partially address the environmental and energy challenges posed by fossil fuel use [15], and it can be obtained from the anaerobic digestion (AD) of microalgal biomass [16–18]. The biochemical process of anaerobic digestion consists of the decomposition of organic matter in the absence of oxygen, resulting in the production of biogas. The process encompasses four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each stage is facilitated by specific microbial consortia that convert complex organic substrates into simpler compounds, culminating in methane production [19]. Biogas is a mixture primarily composed of methane (CH_4) and carbon dioxide (CO_2) [20]; it can be upgraded to yield high-purity methane. The resulting biomethane may be utilized across a range of applications, including heat and power generation, methanol production via liquefaction, transportation fuel through compression, or direct injection into natural gas distribution networks [21].

1.2. An Overview on Microalgae and Cyanobacteria

Microalgae are water-dwelling unicellular photosynthetic microorganisms (of dimensions in the order of micrometres), pertaining to the *Protista* group, with a high reproduction rate. They are considered the oldest living organisms, and they are characterised by no roots, stems, or leaves, nor any coverage of cells around their reproductive cell walls. Therefore, their photosynthetic activity is resultantly more efficient than that in higher plants (theoretically 12.6% higher) [22]; it occurs via chlorophyll a, their key photosynthetic pigment. There are many possibilities for sub-grouping microalgae, such as based on their main pigments (i.e., red, green, diatoms), the compounds they use to store energy, and their cell wall structure [23]. Because of their aquatic habitat, they can easily access water, nutrients, carbon dioxide, etc., effectively supporting their life cycle. Moreover, they can grow both as autotrophs (by using light and inorganic carbon as the energy source for their photosynthetic activity) and as heterotrophs (by using organic carbon sources for energy, like glucose or acetate). Besides their vital role in supporting the steady chemical atmospheric cycle, a variety of different microalgal strains can be employed via different biochemical routes to obtain useful products (and co-products) such as biofuels, chemical compounds, etc. [24,25].

The exploration of microalgal biotechnology began in the mid-20th century and has since evolved significantly. In recent decades, microalgae have gained considerable attention due to their capacity to grow on non-arable land and in different water sources, making them an attractive option for sustainable bioprocessing applications [24]. Nevertheless, one of the main open challenges related to their use at the industrial scale for biofuel production is its economic feasibility, requiring optimisation across cultivation, harvesting, extraction, and product recovery. The conventional downstream processes are often costly and energy-intensive, driving interest in novel technologies and biorefinery-based strategies to improve efficiency and reduce costs [26–28]. Indeed, in addition to bioenergy applications, microalgal biomass can serve as a valuable source of diverse bioactive compounds, which are widely utilised across the agricultural and pharmaceutical sectors. Various species, such as *Chlorella vulgaris*, *Scenedesmus* spp., *Arthrospira platensis*, *Dunaliella*, *Porphyridium cruentum*, *Haematococcus pluvialis*, and *Spirogyra* spp. have been employed for the extraction of proteins, polysaccharides, polyunsaturated fatty acids, pigments, vitamins, and immunomodulatory agents [29].

Cyanobacteria (or blue-green algae) were initially considered to belong to the microalgae group. However, they are prokaryotic photoautotrophs, even if they share many functional traits with microalgae, including the presence of chlorophyll a [30]. For this reason, usually, they are enclosed under the broader term microalgae when discussing biomass technologies [31]. Unlike eukaryotic microalgae, they do not contain a nucleus or other membrane-bound organelles [32]. Moreover, presenting a simple genome allows researchers to investigate how to modify their genome to optimise the production of useful constituents, depending on their industrial use [33,34].

Microalgae and cyanobacteria are increasingly regarded as promising feedstock for the third (and fourth) generation of biofuels and different value-added co-products due to their several advantages over terrestrial crops. Moreover, they can achieve more effective greenhouse gas mitigation compared to conventional land-based crops owing to their superior carbon fixation efficiency and continuous growth potential [29]. Notably, they exhibit rapid growth rates, with many species capable of doubling their biomass within a 24 h period, enabling high productivity over short cultivation cycles. However, their growth depends on many factors, such as temperature, pH, light intensity, nutrient availability, carbon dioxide concentrations, and microalgal strain [35–37]. Nevertheless, their growth rate enables their continuous cultivation during the entire year. Their ability to grow using minimal nutrient inputs makes them well suited to large-scale cultivation, even in resource-constrained environments. Furthermore, many species can efficiently utilise wastewater as a growth medium, which not only supplies essential nutrients but also significantly reduces the reliance on freshwater resources, aligning with sustainable production goals [11,38].

Microorganisms are able to accumulate significant quantities of biomolecules such as lipids, carbohydrates, and proteins, depending on the species and cultivation conditions. All of these lipids are of major interest for biodiesel production due to their high energy content and suitability for transesterification [39]. Carbohydrates, including starch and structural polysaccharides, serve as substrates for bioethanol or biogas production via fermentation and anaerobic digestion processes [40]. In parallel, the protein-rich biomass from certain microalgae offers potential in the feed and food industries, especially when cultivated under nutrient-replete conditions [41]. Additionally, microalgae synthesise high-value compounds such as pigments (e.g., chlorophyll, phycocyanin), polyunsaturated fatty acids, and antioxidants like astaxanthin, which are increasingly exploited in the pharmaceutical, cosmetic, and nutraceutical sectors [41]. This biochemical diversity underpins the concept of a microalgal biorefinery in which biofuel production is integrated with the extraction of co-products to improve its economic viability [40].

Commercial-scale microalgae cultivation is primarily achieved through two main systems: open pond cultures and closed photobioreactors. Open ponds, such as raceway designs, are widely adopted due to their low operational costs, though they are more susceptible to environmental fluctuations. On the other hand, closed photobioreactors offer better control over the growth conditions, albeit with higher capital and maintenance requirements [41]. The microalgae harvesting method is largely influenced by species-specific characteristics, including cell size, biomass density, and surface charge. These physical and biological traits determine the efficiency of processes such as sedimentation, centrifugation, flocculation, and filtration. Additionally, the intended downstream application guides the selection of the harvesting techniques to ensure the integrity and quality of the final product [42]. Subsequently, the microalgal biomass must be processed to prevent natural degradation, as the high water content can accelerate microbial decomposition and lead to product losses. Efficient water removal (dewatering or drying) is essential both to preserving the biomass's integrity and to improving the energy balance of downstream processing. Moreover, residual moisture can significantly increase the processing costs and reduce the overall product yield. Therefore, dewatering and cell disruption are critical initial steps in biomass processing, particularly when targeting intracellular compounds such as lipids or pigments [11,43]. Depending on the biomass composition obtained and the selected processing technology, microalgal biomass can be transformed into biodiesel via lipid transesterification, bioethanol through carbohydrate fermentation, or biogas via anaerobic digestion. Additionally, certain strains have shown potential for producing biohydrogen under specific metabolic conditions, making microalgae a promising platform for multiple renewable energy pathways [44,45].

1.3. Considerations in Terms of Refrigeration

In addition to sustainable fuel production needs, refrigeration is also causing concerns in terms of its emissions and energy requirements; indeed, refrigeration has many applications, from household refrigerators to industrial freezers, as well as cryogenics and air conditioning. In the last decade, the impact of refrigeration on industry, lifestyle, agriculture, and settlement patterns has grown with the rapid growth of mechanical refrigeration technology [46].

Refrigeration is the technique of cooling a control volume and its contents to a lower temperature below the ambient temperature and maintaining this condition. To do so, the removed heat must be rejected into the environment at a higher temperature. Heat is removed from a low-temperature reservoir and transferred into a high-temperature reservoir. This flux occurs against the spontaneous flow dictated by the Second Law of Thermodynamics, so work is required to obtain the cooling effect. For the achievement of this inverse cycle, the working fluid, the refrigerant, plays a fundamental role because of the required properties related to the thermodynamic cycle itself, including its toxicity, its flammability, and its contribution to ozone depletion and climate change. Indeed, from a thermodynamic viewpoint, the refrigerant must undergo a repeated phase transition from a liquid into a gas and back again [47].

The main systems adopted to obtain a refrigeration effect are vapour compression systems. However, there are also some applications with absorption systems [48]. The first type is the most widely spread type of system and needs high-grade energy (mechanical, to move the compressor) to produce the desired output. In vapour absorption refrigeration systems, the mechanical process that occurs within the compressor in traditional cooling systems is replaced by a chemical process [49].

Additionally,in 2006, a regulation concerning fluorinated greenhouse gases (GHGs) was introduced by the European Union (EU) to promote the utilisation of natural refrigerants, gradually eliminating refrigerants with the highest global warming potential (GWP) and ozone depletion potential (ODP), starting in 2011 [50]; this regulation was then revised to form the European Climate Law, which mandates more robust climate action. Indeed, the F-gas Regulation (EU) 2024/573 was enacted on 7 February 2024 and initially came into effect on 11 March 2024. To provide a technical solution in line with the EU requirements, the following main natural refrigerants are currently being promoted [51]:

- Ammonia (R-717): This was already introduced into the refrigeration sector in the 1930s due to its excellent efficiency in refrigeration processes. It features a notably low boiling point and a high energy efficiency as a result of its significant latent heat of evaporation. Despite these advantages, ammonia presents certain limitations: it is toxic at concentrations exceeding 300 ppm, with a low degree of flammability–L2-type–

and is corrosive to copper components. To mitigate this last drawback, ammonia-based systems are often designed to operate with an intermediate (secondary) fluid.
- Carbon dioxide (R-744): This is regarded as a low-environmental-impact refrigerant relative to the substances employed in earlier refrigeration systems. Indeed, it exhibits an ozone depletion potential (ODP) of 0 and a global warming potential (GWP) of 1, indicating its minimal environmental impact. Indeed, it has no adverse effects on the ozone layer. Additionally, R-744 is non-flammable and can be effectively utilised as a secondary refrigerant in various cooling applications.
- Water (R-718): This refrigerant is both non-toxic and non-flammable, with zero impact on ozone layer depletion and global warming potential. Additionally, it is relatively economical compared to many alternative working fluids.
- Hydrocarbons (HCs): These represent a diverse group of refrigerants, including alkanes, alcohols, ketones, and ethers, e.g., isobutane (R-600a) and propane (R-290). Their key benefits stem from their favourable thermophysical characteristics and the absence of halogens like fluorine or chlorine, thereby eliminating the risk of acid generation. Nonetheless, their significant flammability poses safety challenges in certain applications.

There are some technical issues to be solved in relation to the use of natural fluids; however, these solutions must be considered in order to try to reduce the anthropic impact on the environment. Consequently, new or improved systems and technologies must also be introduced, such as absorption systems: Ferdinand Carré created the first gas absorption refrigeration system in 1859 by using gaseous ammonia dissolved in water.

The absorption refrigeration cycles have been deeply analysed, in particular as regards the second law viewpoint [52–54] and their applications in all sectors, from households to power plants [55], coupled with geothermal energy [56,57], solar energy [58,59], and also recovering heat from industrial processes [60–62]. Moreover, these systems have also been introduced in theoretical studies and subsequent prototypes in vehicle and truck air conditioning [63,64] but also in the naval sector [65,66]. Thus, absorption refrigeration systems are still considered an attractive alternative to conventional refrigeration systems because they can be powered using wasted heat or low-grade heat. Indeed, comparing the absorption refrigeration cycle with vapour compression refrigeration, the energy-intensive mechanical compression of the refrigerant in the compression cycle is substituted by a physical–chemical process by using power in the heat flux form at low temperatures [67].

The main difference that allows the use of a different cycle is the refrigerant fluid adopted, which implies different thermochemical properties. Indeed, in absorption systems, a binary solution is used. These two fluids have different saturation temperatures; the one with the lower saturation temperature acts as the refrigerant, while the other is an absorbent, which absorbs the refrigerant [68]. There are several possible working fluids for this cycle, but the most common pairs of refrigerant–absorbent are ammonia–water and lithium bromide–water (Li-Br). The Li-Br couple offers a high performance coefficient and the easiest management. Nevertheless, this solution presents higher costs, working temperature restrictions, corrosion, and crystallisation issues. So, in industrial processes, the water–ammonia solution is preferred. Concerning absorption refrigeration systems, we will focus our attention on one of the components of this solution: ammonia. Indeed, the current pathway used to obtain ammonia is no longer considered viable from an environmental and energetic viewpoint.

1.4. Considerations in Terms of Ammonia

Nowadays, ammonia is the second most produced chemical worldwide [69], particularly for use in the fertiliser and refrigeration sectors. Due to its favourable characteristics, it has also gained attention as a potential solution for renewable energy storage, transport, and utilisation. Additionally, ammonia is increasingly viewed as a practical carrier for hydrogen energy, and its production from microalgae is a promising field of research [70]. The traditional pathway for producing ammonia is the Haber–Bosch process, which represented a milestone during the XX century [71], enabling the synthesis of ammonia using N_2 from the air and H_2 obtained from natural gas. However, the overall process requires high temperatures (up to 600 °C) and pressures (up to 30 MPa). Thus, it presents a drawback in being energy-intensive: this process requires more than 30 GJ of energy for every tonne of ammonia produced [72]; therefore, it accounts for 2% of the fossil energy use worldwide [73]. Moreover, high carbon dioxide equivalent emissions are associated with the Haber–Bosch process. Indeed, the release of about 2.16 kg of carbon dioxide equivalent occurs per kilogram of ammonia produced [72]. Ammonia obtained from this process is classified as brown (or grey) ammonia [74]. The other two ammonia production classifications are blue and green ammonia [74].

Blue ammonia is obtained through the reforming of hydrocarbon feedstocks, integrating carbon capture, utilisation, and storage (CCUS) technologies within ammonia synthesis facilities on site to effectively sequester the resultant CO_2 emissions [75]. An alternative CCUS approach involves the cryogenic separation technique, which selectively removes CO_2 from gas mixtures, such as syngas, facilitating its subsequent utilisation or storage [76], enabling the direct production of liquid CO_2. The captured CO_2 can then be stored using various methods and utilised in a range of production processes [77]. Concerning green ammonia, the production process aims to minimise or entirely eliminate CO_2 emissions. To do so, renewable feedstocks are utilised alongside reduced energy consumption. Currently, the most sought-after yet costly method for producing green ammonia involves generating hydrogen through water electrolysis, which is powered by renewable energy sources such as solar, wind, hydroelectric, and geothermal energy. This technique is commonly referred to as electrochemical ammonia synthesis [72].

Consequently, more sustainable solutions for ammonia production are required [78]. In this context, more sustainable processes can be implemented by exploiting microorganisms to co-produce ammonia, which can be used as a chemical and a refrigerant but also as an energy carrier, as analysed in depth by Nurdiawati et al. [79].

1.5. Bio-Based Ammonia Production

Biological methods are considered environmentally friendly since they use natural processes that do not produce harmful by-products. There are various techniques available for the biological production of ammonia, including [74]

- Biological nitrogen fixation using nitrogenase: Biological nitrogen fixation represents a critical natural process that facilitates the conversion of atmospheric molecular nitrogen into ammonia. This metabolic pathway is responsible for approximately 50% of the bioavailable nitrogen available to support various life forms [80]. Nitrogen-fixing microorganisms, which display remarkable resilience and adaptability, have been extensively studied and utilised in the development of the biofertilisers produced at the commercial scale [81].
- Cell and metabolic engineering for ammonia production: Engineered microorganisms can efficiently ferment various types of biomass, including food waste, microbial biomass, and protein-rich crop residues, owing to the comprehensive understanding of their metabolic pathways. This method utilises microbial fermentation to transform diverse organic materials into valuable nitrogen-rich compounds, thereby supporting sustainable agricultural practices and effective waste management strategies [74].

- Ammonia from wastewater treatment plants: Microbial fuel cell technology can generate ammonia during the treatment of wastewater by using specialised bacteria for ammonia oxidation. In this method, wastewater is directed into an anaerobic anode chamber, where bacteria decompose organic materials, releasing electrons. These electrons then help the ammonia-oxidising bacteria convert ammonia into N_2, effectively cleaning the wastewater [82].
- The hyper-ammonia-producing bacteria route: Several studies in ruminant animal sciences have aimed to enhance microbial protein synthesis and regulate ammonia production [83]. Researchers identified a group of bacteria known as hyper-ammonia-producing bacteria that generate more ammonia than can be utilised by ruminal microbes for functions like protein synthesis [84]. These bacteria convert dietary protein into surplus ammonia during the digestive process, breaking down nitrogen-containing amines into ammonia or ammonium [85]. Recently, the production of biological ammonia through the fermentation of protein biomass by rumen bacteria was explored as a relatively novel approach, highlighting its potential to enhance the bioproduction of ammonia [74].

In this context, the concept of the co-production of fuels, chemicals, etc., from biomass is attracting interest to allow economies of scale to enter the market. A key factor in the achievement of a successful bio-based economy is the development of integrated biorefinery systems with high efficiency and cost-effective processes. An example is the design of the process for achieving the co-production of ammonia with methanol or methanol and urea [86].

1.6. The Aim of This Paper

In this paper, the cultivation of microalgae and cyanobacteria in abandoned pit-lakes from closed mines and quarries is considered as a sustainable way to co-produce biomethane and ammonia. Indeed, when a quarry or a mine is closed, pit-lakes become powerful liabilities, progressively evolving into detrimental states of abandonment [87]. Nonetheless, these lakes can represent the opportunity to provide productive new end uses, depending on their position, conformation, water quality, etc. This can lead to addressing their residual closure risks and mitigating the environmental cost of mining activities.

This approach may also be useful in the context of a circular economy based on local production and the reuse of waste, including the opportunity to create new local employment, even if the latter topic is not a subject of investigation in this paper.

2. Materials and Methods

In this section, an analysis of the theoretical production yield is developed by considering the cultivation of microalgae and cyanobacteria for the co-production of biomethane and ammonia via anaerobic digestion, exploiting the water available in abandoned pit-lakes in dismissed mines or quarries in Alessandria Province (the Piedmont Region, Italy) as the cultivation water. In particular, to determine the theoretical methane and ammonium yields from anaerobic digestion, the composition of the organic matter examined must be known [88].

The maximum potential yields can be obtained by considering a theoretical approach (neglecting the requirements for cell maintenance and anabolism) to the chemical reaction, as summarised by Sialve et al. [89]:

$$C_aH_bO_cN_d + \left[\frac{4a+3d-b-2c}{4}\right] H_2O \longrightarrow \left[\frac{4a-3d+b-2c}{8}\right] CH_4 + \\ + \left[\frac{4a+2c+3d-b}{8}\right] CO_2 + d\, NH_3 \tag{1}$$

The specific theoretical methane yield ($P^*_{CH_4}$), expressed in [$L_{CH_4} g_{TS}^{-1}$], can be evaluated as [89,90]

$$P^*_{CH_4} = \frac{\tilde{V}_{CH_4}}{8} \cdot \left(\frac{4a+b-2c-3d}{12a+b+16c+14d}\right) \tag{2}$$

where $\tilde{V}_{CH_4}$ is the normal volume of CH_4.

The specific ammonia production yield, APY^* [mg g_{TS}^{-1}], can be estimated by using Equation (1), as follows [89]:

$$APY^* = 17000 \cdot \frac{d}{12a+b+16c+14d} \tag{3}$$

Furthermore, once the considered organic matter's composition is known, the higher calorific value, HHV, from the biomass can be estimated using the Du Long equation [90], in values expressed in [MJ kg_{VS}^{-1}]:

$$HHV = \frac{34.1\, C + 102\, H - 9.85\, O + 6.3\, N + 19.1\, S}{100} \tag{4}$$

where C, H, O, N, and S are the carbon, hydrogen, oxygen, nitrogen, and sulphur biomass weight percentage contents in the analysed microorganism.

To obtain a set of preliminary data on the theoretical methane yield and the ammonia production from microalgae and cyanobacteria via anaerobic digestion, their global average composition can be considered as reported by Angelidaki and Sanders [88], where the subscript values are reported in Table 1:

- Proteins: $C_wH_xO_yN_z$;
- Carbohydrates: $C_wH_xO_y$;
- Lipids: $C_wH_xO_y$.

Table 1. Global average composition of microalgae and cyanobacteria [88].

Compound	Subscript in Chemical Formula			
	w	x	y	z
Proteins	2.5	3.5	1.0	0.5
Carbohydrates	6.0	10.0	0.5	–
Lipids	57.0	104.0	6.0	–

Once the mean composition and the mean productivity of each microalgal/cyanobacterial species are known, as is the pit-lake's exploitable water volume, the maximum theoretical methane yield and ammonia production can be calculated for any species of microorganism. This leads to the maximum production yield of biofuel and useful chemicals, such as ammonium, which can also be used in absorption cooling systems more sustainably.

The values have been calculated by considering the following:

1. The specific theoretical yields:
    - $P^*_{CH_4}$ (Equation (2)),
    - APY^* (Equation (3)),

for each average component of the biomass (proteins–carbohydrates–lipids and proteins, respectively);
2. The mean average composition of each i-th strain ($\overline{prot}_i$, $\overline{lip}_i$, $\overline{carb}_i$), using the data from Li et al. [14];
3. The mean average total solid composition of each i-th strain, using the data from Li et al. [14];
4. The mean average volatile solid composition of each i-th strain, using the data from Li et al. [14];
5. The mean biomass productivity ($\bar{p}_i$), using data from Li et al. [14];
6. The time frame considered ($\tau = 1$ yr);
7. The mean available volume of water for each j-th pit-lake in the Alessandria district ($V_{w,j}$), using data from Castagna et al. [91].

Therefore, numerical evaluations, based on the existing data, have been made to provide initial estimates of the theoretical methane yield and ammonia production that can be achieved using various strains of microalgae and cyanobacteria. This takes into account the average composition and biomass productivity values found in the literature regarding microorganisms cultivated in wastewater, as discussed by Li et al. [14].

3. Results

In this section, the preliminary theoretical calculated values are introduced concerning the yields of biomethane and the ammonia co-production from anaerobic digestion by microorganisms, considering the water from abandoned pit-lakes in the Alessandria district as a case study.

The theoretical specific yield values for each main microorganism's component—calculated using Equations (2) and (3)—are

- The theoretical methane yields, $P^*_{CH_4}$ $\left[L_{CH_4}\ g_{TS}^{-1}\right]$, are the following: 0.496 $L_{CH_4}\ g_{TS}^{-1}$ for proteins, 0.415 $L_{CH_4}\ g_{TS}^{-1}$ for carbohydrates, and 1.015 $L_{CH_4}\ g_{TS}^{-1}$ for lipids;
- The theoretical ammonium production APY $\left[mg\ g_{TS}^{-1}\right]$: 150 mg g_{TS}^{-1} for proteins.

These values have been calculated using Equation (3) following the theoretical approaches summarised by Sialve et al. [89] and Heaven et al. [90], considering the main composition of proteins, carbohydrates, and lipids given in the previous section.

Performing the calculations, the first hypothesis introduced is related to biomass productivity; indeed, following Nwoba et al. [92], the mean value from the biomass productivity data obtained in photobioreactors given by Li et al. [14] has been treated by dividing it by a factor of 2.1, accounting for the differences in growth between photobioreactors and open ponds. This factor was determined by comparing the growth of the same strain of microalgae in both systems. In photobioreactors, the mean productivity of this particular culture is 2.1 times greater than that in open ponds, where the productivity is limited by external environmental conditions [92].

Now, introducing the average values of the percentage composition of proteins, carbohydrates, and lipids for certain microalgal and cyanobacteria strains reported by Li et al. [14] and the average biomass productivity $\bar{p}_i$ for each i-th strain, it is possible to calculate the HHV by using Equation (4), as reported in Table 2.

As concerns the cultivation water, the exploitation of seven abandoned pit-lakes in the Alessandria district (the Piedmont Region, Italy) has been hypothesised. The data related to these lakes are summarised in Table 3, as reported by Castagna et al. [91]. These lakes have been selected because they are in the same local territory (Alessandria Province) and present suitable nutrient and environmental conditions for microorganism growth [91].

Table 2. The mean biomass productivity (p) and biomethane higher calorific value (HHV) calculated by using the average percentage composition data [14] for certain microalgal and cyanobacteria strains and Equation (4).

Strain	p [g L^{-1} d^{-1}]	HHV [MJ kg^{-1}]
Arthrospira maxima	0.23	18.72
Arthrospira platensis	2.18	17.13
Botryococcus braunii	0.02	14.37
Chlamydomonas rheinhardii	1.41	20.81
Chlorella spp.	1.26	24.78
Chlorella pyrenoidosa	0.525	23.21
Chlorella vulgaris	0.11	25.47
Haematococcus pluvialis	0.055	22.12
Isochrysis galbana	0.915	17.31
Scenedesmus obliquus	0.039	21.74

Subsequently, it is possible to calculate, for each strain considered and previously summarised, the annual ammonium production (Table 4) and the annual methane yield (Table 5) by using Equations (3) and (2), as well as the average composition of each strain, the specific yield per component, the mean biomass productivity per strain considering an open cultivation system (pit-lake), and the cultivation volume.

Table 3. The pit-lakes considered in this study and their main geometrical characteristics, as well as their identification numbers, were retrieved from Castagna et al. [91].

Quantity	Pit-Lake ID Number						
	9	15	17	18	19	20	21
Volume [$\cdot 10^7$ L]	1300	170	190	120	100	150	6
Average depth [m]	8	18	28	18	12	8	10

Table 4. The maximum average theoretical ammonia release during anaerobic digestion (APY), expressed in [t yr^{-1}], calculated using Equation (3) and the average percentage composition values [14] for certain microalgal and cyanobacteria strains [14]. The numbers at the bottom represent the pit-lake identification numbers, as presented by Castagna et al. [91].

Strain	APY [t_{NH_3} yr^{-1}]						
	Pit-Lake ID Number						
	9	15	17	18	19	20	21
Arthrospira maxima	80.94	4.70	3.38	3.32	4.15	9.34	0.30
Arthrospira platensis	583.32	33.90	24.36	23.93	29.91	67.31	2.15
Botryococcus braunii	2.36	0.14	0.10	0.10	0.12	0.27	0.01
Chlamydomonas rheinhardii	363.65	21.13	15.19	14.92	18.65	41.96	1.34
Chlorella spp.	368.97	21.44	15.41	15.14	18.92	42.57	1.36
Chlorella pyrenoidosa	165.58	9.62	6.91	6.79	8.49	19.11	0.61
Chlorella vulgaris	32.21	1.87	1.35	1.32	1.65	3.72	0.12
Haematococcus pluvialis	14.18	0.82	0.59	0.58	0.73	1.64	0.05
Isochrysis galbana	132.74	7.71	5.54	5.45	6.81	15.32	0.49
Scenedesmus obliquus	7.10	0.41	0.30	0.29	0.36	0.82	0.03

Regarding the microorganisms' growth, the maximum depth considered exploitable in open ponds is about 30×10^{-2} m [93,94]. Therefore, to calculate the volume of water available for cultivation, only the first 15×10^{-2} m of depth was considered. Indeed, the greater the depth exploited, the less solar radiation is absorbed by photosynthetic microorganisms. Moreover, the hypothesised harvesting volume was calculated by using only half of the surface of the pit-lake to allow for continuous reproduction by the microorganisms.

Furthermore, to evaluate the yearly production of methane and the ammonia production, the values obtained through the theoretical calculations using all of the above mentioned hypotheses have been quartered in order to accommodate the consideration that the annual productivity can substantially vary during the year, resulting in lower production during the winter period, due to external conditions. Additionally, to consider the ammonia output, the ammonium value has been multiplied by 0.82, which is the mean value considered for the conversion of ammonium into ammonia, as reported by Grasham et al. [95].

Table 5. The maximum average theoretical methane yield (P_{CH_4}), expressed in [L_{CH_4} yr^{-1}], calculated using Equation (2) and the average percentage composition values [14] for certain microalgal and cyanobacteria strains. The numbers at the bottom represent the pit-lake identification numbers as presented by Castagna et al. [91].

Strain	P_{CH_4} [$\times 10^5$ L_{CH_4} yr^{-1}]						
	Pit-Lake ID Number						
	9	**15**	**17**	**18**	**19**	**20**	**21**
Arthrospira maxima	5492	319	229	225	282	634	20
Arthrospira platensis	48,453	2816	2023	1988	2485	5591	179
Botryococcus braunii	390	23	16	16	20	45	1
Chlamydomonas rheinhardii	38,908	2261	1625	1596	1995	4489	144
Chlorella spp.	41,675	2422	1740	1710	2137	4809	154
Chlorella pyrenoidosa	16,015	931	669	657	821	1848	59
Chlorella vulgaris	3750	218	157	154	192	433	14
Haematococcus pluvialis	1611	94	67	66	83	186	6
Isochrysis galbana	21,456	1247	896	880	1100	2476	79
Scenedesmus obliquus	1154	67	48	47	59	133	4

In relation to the numerical evaluations reported in Tables 4 and 5, it is possible to highlight that the more interesting species for co-production are *Arthrospira platensis*, *Chlamydomonas rheinhardii*, *Chlorella* spp., and *Chlorella pyrenoidosa* because together, they are able to produce the greater amount of NH_3 and CH_4.

4. Discussion

Global thermal measurements have been recorded for 150 years now, and a relative analysis of these data highlights that recent decades have been the hottest [96,97]; additionally, forecasts indicate that the global average temperature could rise by approximately 2–4 °C by 2100. Human activities are likely contributing to global warming. However, even if we were to disregard this human impact, it is undeniable that pollution negatively affects our quality of life because of its health implications and adverse environmental conditions [98–100]. In this context, at the 2017 UN IPCC Conference held in Marrakesh, it was suggested to contain the increase in temperature up to 2°C above pre-industrial levels [96]. Thus, some changes toward effective sustainable development have to be introduced into all human activities. This also means improving the technologies used for power generation and transportation, industrial production, agriculture, and cooling systems. In this context, biofuels and natural fuels are considered a possible resource for responding to the previously cited issues; indeed, they represent a viable alternative [101–103], with the following main characteristics [104,105]:

- Readily accessible: They can be sourced from various types of biomass;
- Technically and ecologically viable: The biomass used for their production comes from photosynthetic organisms that absorb the same amount of carbon dioxide that is released during the combustion of biofuels throughout their life cycle;
- Economically viable: Every country has the potential to locally produce the raw materials needed for biofuel production.

In this context, the Italian regulations must be considered. Indeed, the Ministry of the Environment and Energy Security has developed the National Integrated Energy and Climate Plan (PNIEC 2024) [106], revising the objectives of the previous version (PNIEC 2019) and taking into account the evolution of European energy and climate legislation, as well as the current geopolitical context. It includes a survey of the key energy and emissions indicators to establish the state of the art as of 2021 (the reference year for the development of the new plan) and provides projections for 2030 based on the current policies (the baseline scenario). Furthermore, it introduces a long-term strategy with a time horizon extending to 2050. Thus, the PNIEC 2024 establishes the national objectives for 2030 on energy efficiency, renewable source use, and reductions in greenhouse gas (GHG) emissions, as well as the targets on energy security, interconnections, the single energy market and its competitiveness, and sustainable mobility, outlining for each of these the measures and techniques that will be implemented to ensure their achievement. Moreover, the EU regulations also establish that the voluntary mitigation measures included in the long-term strategy must be able to guarantee carbon neutrality by 2050, taking into account the specificities of the production, energy, and economic and social structure of our country.

Therefore, in alignment with the European Union's binding target of achieving at least a 32% share of renewable energy in the gross final consumption by 2030, as stipulated in Article 3 of Directive (EU) 2018/2001 [107], Italy's PNIEC 2024 updates the national objectives to promote and expand the use of renewable energy sources. This strategic goal complements efforts to reduce GHG emissions and enhance carbon sinks, contributing to the broader decarbonization agenda. Additionally, to facilitate the deployment of renewable energy systems, recent legislative measures have introduced regulatory simplifications. Notably, Law Decree 17/2022 [108] and Law Decree 13/2023 [109] have implemented provisions that

- Define *agrivoltaic systems* as photovoltaic installations with elevated, rotating modules situated no more than 3 km from areas designated for industrial, artisanal, or commercial use;
- Permit the installation of floating photovoltaic systems on artificial water bodies, including disused quarry reservoirs and irrigation channels;
- Identify *suitable areas* for photovoltaic installations, streamlining the authorization processes and promoting development in these zones.

The PNIEC 2024 reinforces Italy's objective of the decarbonization of energy and economic systems to become a regional area with social, economic, and productive dimensions with net zero emissions. However, this path is remarkably complex and does not lend itself to simple solutions or pre-established choices, rather requiring measures capable of promoting the use of all of the available technologies, behaviours, and energy sources capable of decarbonising the country's economy, adapting different choices according to the needs of productive, economic, and social areas. Furthermore, the updated PNIEC 2024 outlines the target of reaching 1×10^{10} m^3 yr^{-1} of biomethane production by 2030, representing a more than tenfold increase compared to the current output, which stands at roughly 0.8×10^9 m^3 yr^{-1}. This objective underscores Italy's strategic focus on scaling up its renewable gas production and consolidating its role as a key contributor to the European biomethane market [106,110].

In order to reach this target, besides the exploitation of agricultural waste and animal manure, the use of microalgae and cyanobacteria to produce biomethane may be considered too, taking into account the advantage of obtaining other by-products, such as ammonia. In this context, the co-production of bioproducts could represent an interesting approach to achieving the main targets introduced in the PNIEC 2024.

In this transition path, it is necessary to combine decarbonisation policies with those aiming to maintain quality of life and social services, fight against energy poverty, and maintain competitiveness and employment, given the structure of the Italian productive and manufacturing fabric, not only considering non-European countries that have still not implemented decarbonisation policies with equal determination and speed but also avoiding the phenomena of intra-European competition, due to national measures that are not harmonised at the community level. It is therefore a question of developing the measures described in this plan in programmatic terms and introducing them into operational tools that together improve energy security, environmental protection, and the accessibility of energy costs, contributing to the European objectives in terms of energy and the environment.

The co-production of ammonia and biofuels could represent a new and interesting approach to supporting this political and environmental aim. Our proposal is the use of dismissed areas (in this paper, we focused on pit-lakes, but this is only an example), often far from inhabited centres. This is fundamental as regards biofuel production due to the consequences of this activity (e.g., odours, fire safety, etc.), which generate hostility and distrust in residents towards accepting biorefineries near their homes.

Furthermore, as for competition with the loss of biodiversity, land-use changes and water depletion [111–113], ethical responsibility, and the energy and cost requirements [114], biofuels and natural fuels must be obtained only by considering sustainable processes. Moreover, the concept of biorefinery, which encompasses other useful products (including chemicals), could improve the overall beneficial effect.

Within this context of reference, the potential of microalgae to produce biomass and reduce greenhouse gas emissions could offer a viable solution for sustainable energy generation. The simultaneous production of biofuels and valuable natural substances from these aquatic resources is gaining traction in the research [95,115], particularly regarding their role in wastewater treatment and the utilisation of contaminated natural resources, as well as the creation of other high-value products that can foster economic growth. Although there are approximately one hundred thousand species of algae on the planet, only around twenty are currently utilised in industrial and economic applications. Macroalgae can generate significant amounts of biomass, estimated between 7–30 t $ha^{-1}yr^{-1}$, but the challenge of cultivating them in offshore environments remains unresolved [116]. In contrast, microalgae thrive in both marine and freshwater settings. They share a photosynthetic process similar to that in terrestrial plants, yet their simpler cellular structure and aquatic surroundings enable them to convert light energy into biomass more efficiently [117,118].

Finally, microalgae offer a promising avenue for producing biomass feedstock to generate biofuels [119]. This is attributed to several characteristics in microalgae:

- They can provide a consistent supply of biomass, as their harvesting is not subject to seasonal variations [120];
- They can be cultivated in various water environments, including seawater, freshwater, and wastewater, which leads to reduced freshwater use and the ability to utilise otherwise unproductive areas [121,122];
- They exhibit a rapid doubling time during their exponential growth phase, typically taking less than 3.5 h [39];
- They have a high potential yield relative to the area used for cultivation [123].

Moreover, microalgae and cyanobacteria have been shown to effectively remove pollutants such as ammonium, nitrate, and heavy metals, all while generating biomass suitable for conversion into biofuels and bio-based useful products [124–126]. Such synergistic systems can support a circular bioeconomy model, offering a pathway for sustainable water reuse, pollution mitigation, and renewable energy production.

Therefore, the water bodies available in disused and decommissioned mining sites may offer promising locations for the establishment of algal cultivation systems. The following factors can contribute to their potential suitability:

- These environments usually contain detrimental water and thus are unsuitable for human consumption or agricultural use, making them available for non-potable applications only (e.g., algal biomass production);
- The spatial confinement of mining sites can act as a natural containment system, reducing the risk of spreading invasive algal species into surrounding ecosystems;
- A second life can be offered to these sites, which usually have low market value, presenting an opportunity for repurposing, potentially enabling the development of novel bio-industrial ventures and generating employment at the local level;
- Due to the bioremediation capacity of microalgae and cyanobacteria strains (immobilising or absorbing certain heavy metals), they can contribute to the bioremediation of soils and waters impacted by previous extractive operations;
- Even in underground mining facilities, artificial lighting systems can enhance the photosynthetic activity, while naturally stable temperature conditions may improve the cultivation efficiency.

This study presents a theoretical framework that relies only on the average data from the existing literature. As a result, various constraints associated with the growth condition parameters, pretreatment methodologies, plant design, and operational conditions require empirical evaluations under different operating conditions. This is essential for accurately assessing the actual production and its potential relevance to industrial applications.

5. Conclusions

One of the main issues facing industrialised societies today is the management of carbon dioxide emissions. The primary actions suggested for reducing CO_2 emissions, already present in the Kyoto Protocol, have been

- Using sources of renewable energy.
- CO_2 sequestration, which has significantly high estimated investment costs, making its implementation challenging.
- Encouragement of the current high-efficiency technologies and the integration of advanced energy systems with low CO_2 emissions; in fact, reducing CO_2 is closely linked to the thermodynamic efficiency of a facility, and energy policies could be designed to promote the best available technologies and their adoption.

At the same time, the CO_2 emission problem could represent a concrete opportunity to promote the high-efficiency design of conventional plants and the consequent dissemination of advanced technologies. In 1992, to reduce greenhouse gases (GHGs), the *United Nations Conference on Environment and Development* in Rio de Janeiro underlined the need for a global strategy, including economic instruments such as taxes [127].

Many factors can affect overall GHG emissions: economic growth levels, technological development, and the processes used [127]. As a result, measuring, monitoring, and assessing programmes are essential for determining the effectiveness of sustainable policies, especially concerning reducing emissions.

Utilising biofuels could be a potential way to support sustainable initiatives, with particular regard to biomethane for energy production and domestic uses. Indeed, biofuels could represent an economic opportunity with important environmental consequences concerning reductions in the climate emissions from the transport and energy sectors; in particular, this is interesting if biofuels are co-produced with natural fluids for refrigeration, such as ammonia. Indeed, ammonia presents a great number of industrial applications, both in the refrigeration sector and in the chemical and agro-industrial sectors. Moreover, ammonia production is responsible for 2% of our overall environmental impact; consequently, the co-production of ammonia with biomethane could represent an interesting field of investigation of the feasibility of its contribution to a reduction in environmental impact in accordance with the statements on sustainable development.

In this paper, the co-production of biofuels and natural refrigerants for industrial and domestic uses is proposed as a possible optimisation approach to reducing the environmental impact of methane and ammonia production; a theoretical assessment of the co-production yield from the anaerobic digestion of microalgae and cyanobacteria cultivated in pit-lakes from abandoned mines and quarries was thus carried out, with a focus on the Alessandria district as a case study. This analysis, based on the average data from the literature related to anaerobic digestion, identified *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Chlorella* spp., and *Chlorella pyrenoidosa* as the most promising species in terms of their biomethane and ammonia yields.

Future developments could be related to certain variables—such as the growth conditions, light availability, temperature, nutrient concentrations, and the detailed specific physico-chemical properties of the pit-lakes. Therefore, these results should be regarded as a preliminary exploration rather than a feasibility assessment.

Nonetheless, the concept of repurposing mining pit-lakes for microalgal and cyanobacterial cultivation offers a potentially sustainable strategy for resource recovery. These lakes, often regarded as post-mining liabilities, can instead be seen as underutilised assets capable of supporting bio-based production systems. By providing productive new end uses, such approaches may reduce the residual environmental risks of mine closures and contribute to circular economy objectives.

Future research should focus on experimental validation of biomass's productivity under real environmental conditions, evaluation of the water quality and lake morphology, and the development of integrated biorefinery models. Such efforts will be essential for moving from theoretical assessments to practical implementation, paving the way for innovative and sustainable uses of post-industrial landscapes.

Author Contributions: Conceptualisation: U.L.; methodology: U.L. and G.G.; validation: U.L. and G.G.; formal analysis: U.L. and G.G.; investigation: U.L. and G.G.; resources: U.L.; data curation: G.G.; writing—original draft preparation: U.L. and G.G.; writing—review and editing: U.L. and G.G.; visualisation: G.G.; supervision: U.L.; project administration: U.L.; funding acquisition: U.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the sources to obtain all raw data used to perform the calculations are quoted along the text.

Conflicts of Interest: The authors declare no conflicts of interest.

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