Startup Aid
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Turning CO_2 into Protein-Rich Livestock Feed Using CRISPR-Engineered Anaerobic Bacteria

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1 The Science Driving It

In the face of accelerating climate change and rising global demand for sustainable sources of animal feed, **Startup Aid** presents an innovative and environmentally responsible solution. The core aim of our project is to **extract excess CO_2 from polluted air** and transform it into **protein-rich biomass** suitable for livestock consumption, particularly for animals such as pigs and poultry.

Our approach integrates two powerful technologies:

1. **Direct Air Capture (DAC)** using hydroxides like **calcium hydroxide ($Ca(OH)_2$)** to chemically trap atmospheric CO_2.

2. **CRISPR-based genetic engineering** applied to **anaerobic, CO_2-fixing bacteria**, which enables them to efficiently convert the captured carbon into **valuable proteins** through enhanced metabolic pathways.

By leveraging the **Wood–Ljungdahl Pathway**—a natural carbon fixation process found in anaerobic microbes—and boosting it with CRISPR gene editing, our system can redirect carbon from being a pollutant into becoming a nutritional resource. This not only reduces greenhouse gases but also replaces environmentally taxing protein sources like soy or fishmeal.

2 Documentation of the Prototype

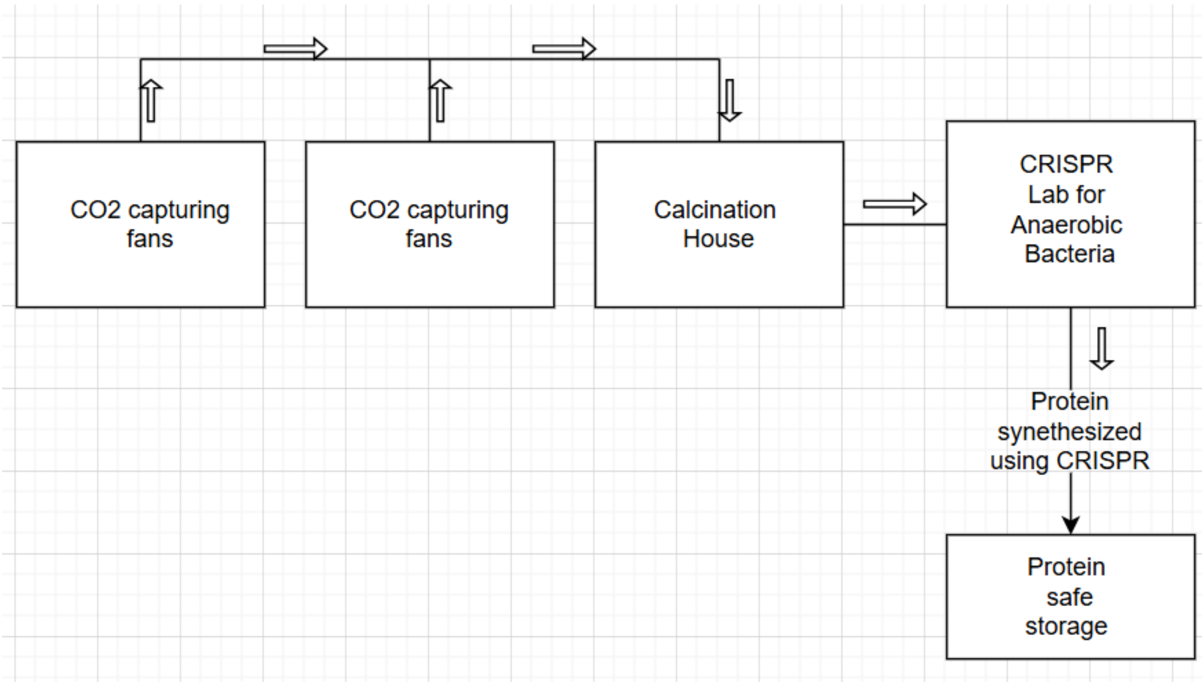

Figure 1: 2D outline prototype of the product

2.1 CO_2 Extraction and Regeneration: Chemistry and Process Flow

Startup Aid's carbon capture system uses a recyclable chemical loop involving calcium hydroxide to extract atmospheric CO_2 and prepare it for conversion into proteins. The process involves three major steps: capture, calcination, and regeneration.

2.1.1 1. Air Intake and CO_2 Capture (Block I)

Polluted air is pulled into a reaction chamber where it passes over surfaces coated with calcium hydroxide. CO_2 reacts with calcium hydroxide to form solid calcium carbonate and water:

$$CO_{2(g)} + Ca(OH)_{2(s)} \rightarrow CaCO_{3(s)} + H_2O_{(l)}$$

This exothermic reaction captures CO_2 as an easily collectable solid.

2.1.2 2. Calcination for CO_2 Regeneration (Block II)

The collected calcium carbonate is sent to a high-temperature calcination unit, where it decomposes into calcium oxide and releases pure CO_2 gas:

$$CaCO_{3(s)} \rightarrow CaO_{(s)} + CO_{2(g)}$$

This CO_2 is captured and routed into bioreactors for microbial protein production.

2.1.3 3. Recycling Calcium Oxide

The calcium oxide produced in calcination is then hydrated with water to regenerate calcium hydroxide:

$$CaO_{(s)} + H_2O_{(l)} \rightarrow Ca(OH)_{2(s)}$$

This allows the system to reuse the calcium-based absorbent in a sustainable cycle.

This chemical loop is central to the CO_2 supply for downstream biological conversion in our system.

2.2 CRISPR-Enabled CO_2 Conversion to Protein-Rich Biomass Using Anaerobic Bacteria

1. Overview of the Biological Conversion System

Startup Aid utilizes **CRISPR-Cas9 gene editing** technology to enhance the metabolic pathways of anaerobic, CO_2-fixing bacteria for the efficient conversion of carbon dioxide into protein-rich biomass. This biomass serves as a sustainable and nutritious feed supplement for livestock such as pigs and poultry.

2. Choice of Bacterial Strains

Anaerobic autotrophic bacteria capable of CO_2 fixation include:

- *Clostridium ljungdahlii*
- *Acetobacterium woodii*
- *Moorella thermoacetica*

These bacteria utilize the **Wood–Ljungdahl Pathway** for carbon fixation in anaerobic environments, converting CO_2 and H_2 into acetyl-CoA, which is further processed into biomass.

3. CO_2 Fixation via Wood–Ljungdahl Pathway

The primary carbon-fixing reaction is as follows:

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

Here, acetic acid (CH_3COOH) serves as a precursor to acetyl-CoA.

4. Engineering with CRISPR-Cas9

CRISPR is used to:

- **Upregulate** genes involved in CO_2 fixation: *fdh*, *cooS*, *acs*.
- **Introduce** new biosynthetic pathways for essential amino acids (lysine, methionine, tryptophan).
- **Knock out** competing metabolic pathways that divert carbon from protein biosynthesis.

5. Protein Biosynthesis Pathway from CO_2

Step 1: Fixation of CO_2 to Acetic Acid

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$$

Step 2: Formation of Acetyl-CoA

$$CH_3COOH + CoA + ATP \rightarrow CH_3CO–CoA + AMP + PPi$$

Step 3: Amino Acid and Protein Synthesis

$$Acetyl - CoA + NH_4^+ + ATP \rightarrow Aminoacids \rightarrow Polypeptides(Proteins)$$

These proteins accumulate as cellular biomass, forming a nutritious, high-protein product for animal feed.

6. Final Output and Benefits

- Biomass contains 60–80% protein.
- Rich in essential amino acids required in livestock diets.
- Safe, scalable, and produced independently of agriculture or sunlight.

7. Scientific and Industrial Validation

Supporting research:

- Liew et al. (2017): Metabolic engineering of *Clostridium autoethanogenum* for CO_2-based bioproduction.
- Molitor et al. (2019): Pathway optimization in acetogens for sustainable feedstock conversion.
- Heffernan et al. (2020): Use of single-cell proteins (SCP) as sustainable feed.

Real-world companies:

- **Calysta** (FeedKind® protein)
- **Solar Foods** (protein from air)
- **Air Protein** (CO_2-based food production)

2.3 Monetary Analysis and Budget Estimate (Pilot Scale)

Assumptions

- Pilot production: ∼10 kg microbial protein per day
- CO_2 captured: ∼25 kg/day
- Operating days/year: 300
- Location: Urban/semi-urban research zone

1. Infrastructure Setup

Component	**Cost (USD)**
CO_2 Capture Block ($Ca(OH)_2$ chambers + fans)	15,000
Calcination Unit (small-scale kiln/furnace)	10,000
Anaerobic Bioreactor System	25,000
CRISPR Lab Setup (PCR, incubator, hood)	20,000
Facility Setup (ventilation, plumbing, electrical)	10,000
Subtotal (Infrastructure)	**80,000**

2. Operational Inputs (Annual)

Item	Cost (USD)
Calcium Hydroxide (5 tons/year)	600
Electricity (reactors, heating)	6,000
CRISPR Kits, Enzymes, Reagents	8,000
Lab Chemicals and Nutrients	5,000
Water and Gas (H_2, N-sources)	2,000
Waste Management and Safety Equipment	2,000
Subtotal (Operational Inputs)	**23,600**

3. Personnel (Annual)

Role	Annual Cost (USD)
Biotech Researcher (Full Time)	30,000
Lab Assistant (Full Time)	15,000
Maintenance Technician (0.5 FTE)	10,000
CRISPR Consultant (Part Time)	5,000
Subtotal (Personnel)	**60,000**

4. Harvesting and Packaging

Item	Cost (USD)
Biomass Dryer and Pelletizer	7,000
Packaging Equipment and Materials	1,000
Subtotal	**8,000**

5. Miscellaneous

Item	Cost (USD)
Licensing, Certification, Audits	3,000
Insurance	2,000
Contingency (5%)	8,200
Subtotal (Miscellaneous)	**13,200**

Total Estimated Budget

Category	Total Cost (USD)
Infrastructure Setup	80,000
Operational Inputs	23,600
Personnel	60,000
Harvesting and Packaging	8,000
Miscellaneous	13,200
Total Budget Estimate	**$184,800**

Note: Pilot plants are not revenue-positive initially. The purpose is to:

- Validate technology
- Attract seed funding or Series A
- Get regulatory approval