EXTRACTION, SYNTHESIS AND CHARACTERISATION OF BIO-SUSTAINBLE SOY-OIL BASED ALGAL INK

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Introduction:

The proposed project, titled "Extraction, Synthesis and Characterisation of Bio-Sustainable Soy-Oil Based Algal Ink", is driven by the pressing need to find environmentally responsible alternatives to conventional petrochemical inks, which are major contributors to pollution due to their high content of volatile organic compounds (VOCs), non-biodegradable nature, and reliance on finite fossil fuels. The motive behind this initiative stems from the increasing global demand for sustainable, non-toxic products that align with environmental safety and public health. As highlighted in the literature, petroleum-based inks emit harmful VOCs during printing and drying, contributing to air pollution, smog formation, and respiratory illnesses, while also causing long-term ecological damage through the accumulation of synthetic, heavy-metal-containing pigments in landfills and water bodies.

In response to these challenges, this project seeks to develop a bio-based ink using renewable, plant- and algae-derived materials—specifically, soybean oil as a carrier an d phycocyanin extracted from Spirulina sp. as the primary pigment. The formulation also incorporates gum Arabic as a natural binder, Butylated Hydroxy Toluene (BHT) as an antioxidant, and iron alum as a mordant to enhance print quality and adhesion. These choices are informed by extensive literature supporting the environmental advantages of soy-based inks, which include lower carbon footprints, reduced VOC emissions, biodegradability, and compatibility with circular economy models.

Furthermore, phycocyanin offers a vibrant, natural blue pigment with antioxidant and antimicrobial benefits, positioning it as a safe and effective alternative to synthetic dyes. This project is significant not only for its contribution to greener printing technologies but also for its potential to reduce industrial dependence on petrochemicals, promote agricultural and algal resource utilization, and pave the way for future innovations in sustainable ink development.

Objectives:

- Develop and optimize a synthesis process for creating bio-sustainable algal inks
- Implement and refine extraction techniques to obtain high yield soya oil and algal pigment
- Conduct comprehensive characterization of soy-oil based algal-ink including viscosity, drying time and optimize the formulation to enhance properties of ink like stability, durability, adhesion
- Evaluate sustainability by assessing biodegradability, toxicity
- Optimize the formulation to enhance properties of ink like stability, durability, adhesion
- Further develop the product into a lithographic ink suitable for high temperature printing application

Methodology:

1. Soybean Oil Extraction

- **Raw Material Preparation:**
  - 200g of soybeans were roasted at 80–100°C to loosen the hulls.
  - Dehulling was done manually, followed by mechanical grinding to obtain a fine soybean powder.
- **Soxhlet Extraction:**
  - Two solvents were tested—ethanol and hexane.
  - Ethanol extraction yielded lower oil volume and required longer processing.

- Hexane, being non-polar, offered higher efficiency, with oil yields of 22–29 mL per batch(200gm of powdered

Figure 5: Soxhlet extraction apparatus

Figure 6: Oil-solvent mixture obtained after soxhlet extraction

  soybeans) in 4–6 hours at 75–85°C.
- The Soxhlet setup included a thimble filled with ground soybeans placed in the extraction chamber, connected to a condenser and a round-bottom flask.

- **Purification:**
  - The oil-solvent mixture underwent simple distillation at 85°C for 4.5 hours.
  - Hexane was effectively removed and recovered for reuse.
  - The resulting purified soybean oil was stored for ink formulation.

The oil-solvent mixture obtained was then subjected to simple distillation at 85°C for 4-5 hours, effectively removing hexane and recovering it for reuse. The purified soybean oil was thus obtained.

Figure 1 Roasting of soyabeans

Figure 2 Roasting of soya beans in bigger batches

Figure 3 Dehulling of soyabeans

Figure 4 Sieving

Figure 7: Supernatant obtained using 10% NaCl

Figure 8: Supernatant obtained using 100mM phosphate buffer (pH 7)

1. **Phycocyanin Pigment Extraction:**

- **Hydration of Biomass**:
  - Dried *Spirulina sp.* (*Arthrospira platensis*) biomass (6–8g) was hydrated in 40 mL phosphate buffer (100 mM, pH 7) to create 6%,15% and 20% w/v suspensions.
- **Extraction Process**:
  - The hydrated biomass was subjected to mechanical agitation at 85 rpm for 4 hours at 29–30°C.
  - This disrupted cell membranes and released intracellular phycocyanin.
- **Centrifugation**:
  - The mixture was centrifuged at 8000 rpm for 10 minutes at 4°C in a cold centrifuge.
  - The pigmented supernatant (crude extract) was collected for use.
- **Spectrophotometric confirmation of Pigment**:

- The pigment was confirmed and quantified by spectrophotometry of sample dilutions (1:24) in water in the visible range. The peaks were observed at 260nm and 620nm indicating presence of proteins and C-phycocyanin respectively
- The quantification was done using the formula:

$$\gamma(\text{C-PC}) = \frac{A_{615\,\text{nm}} - 0.474\, A_{652\,\text{nm}}}{5.34}$$

- Where A_{615nm} and A_{652nm} represent the absorbance of the solutions prepared are the wavelengths mentioned respectively and γ represents yield of phycocyanin pigment in mg/mL

- **Lyophilization (Freeze-Drying)**:
  - The crude extract was freeze-dried at –88°C for 24–36 hours.
  - However, lyophilized pigment showed phase separation during ink blending.
  - Hence, the **non-lyophilized crude extract** was used directly in ink formulation for better emulsion stability.

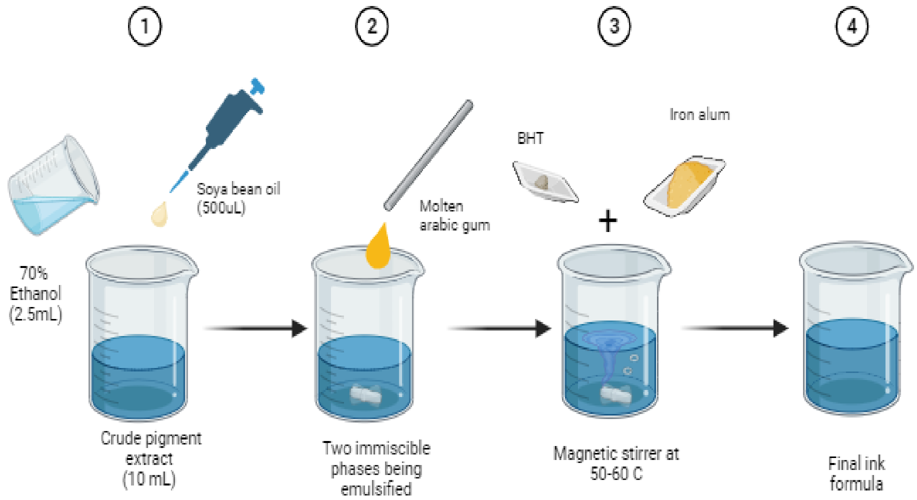
2. **Ink Formulation**:

The ink formulation process was a critical phase of the project, aimed at creating a stable, homogenous emulsion with optimal viscosity, drying time, and color intensity. The following amounts were used for preparation of 20ml ink. The ink was formulated using the following ingredients:

- **Soybean oil (500 µL – 1 mL)**: Acts as the base and carrier medium for dispersing the pigment. Its renewable nature and low VOC emission make it an environmentally preferred alternative to mineral oils.
- **Phycocyanin pigment (10–20 mL)**: Provides a deep natural blue **color**, sourced from *Spirulina*, with additional antioxidant and antimicrobial properties.
- **Arabic gum (1-2 mL of 50–60% w/v solution)**: A natural biopolymer that acts as a binder and emulsifier, stabilizing the oil–water emulsion and improving the ink's adhesion and consistency.

- **Ethanol (70%)**: Serves as a solvent to reduce the ink's drying time by enhancing evaporation and improving pigment dispersion.
- **Butylated Hydroxy Toluene (BHT)**: Added at 0.05–0.1 g per batch as an antioxidant to prevent oxidation of the soy oil, ensuring shelf stability.
- **Iron alum (0.25 g)**: Used as a mordant to enhance color fastness and adherence by forming coordination complexes between the pigment and substrate fibers.

Formulation Process:



The formulation was carried out in a temperature-controlled environment at 55°C, using magnetic stirring at 450 rpm for 30 minutes to emulsify the oil and aqueous phases. The temperature ensured proper melting and dispersion of Arabic gum and facilitated uniform blending of all components. The proportions of ingredients were adjusted across multiple trials to identify the most effective combination. It was observed that increasing the biomass concentration to 20% w/v during phycocyanin extraction resulted in a richer and more vibrant pigment, which improved the overall color intensity of the ink.

Table 3: Formula 3

Components	Quantity
Pigment (15% biomass)	10mL
Soyabean-oil	500µL
Ethanol (70%)	2.5mL
Arabic gum	50% w/v (5g in 7mL water)
BHT(Butylated Hydroxy Toluene)	0.1g
Iron alum	0.25g (20% of the total volume)

Table 4: Formula 4

Components	Quantity
Pigment (20% biomass)	14mL
Soyabean-oil	750µL
Ethanol (70%)	2.5mL
Arabic gum	50% w/v (5g in 7mL water)
BHT(Butylated Hydroxy Toluene)	0.1g
Iron alum	0.2g

Table 1: Formula 1

Components	Quantity
Pigment (6% biomass)	1g in 5mL
Soyabean-oil	1mL
Ethanol (70%)	---
Arabic gum	60% w/v (3g in 5mL water)
BHT(Butylated Hydroxy Toluene)	---
Iron alum	---

Table 2: Formula 2

Components	Quantity
Pigment (6% biomass)	4mL
Soyabean-oil	200µL
Ethanol (70%)	1mL
Arabic gum	50% w/v (5g in 7mL water)
BHT(Butylated Hydroxy Toluene)	0.05g
Iron alum	---

The successful formulation resulted in a stable, flowable ink with desirable spreadability, adhesion, and drying characteristics. Emphasis was placed on emulsion stability, ensuring that the oil and water phases did not separate over time, which is a common challenge in bio-based ink systems.

Figure 9 Figure representing synthesis of soy-oil based algal ink

3. **Characterization**

   The formulated ink was subjected to multiple characterization tests to evaluate its performance and environmental safety:

   - **Density** was calculated using standard mass/volume measurement techniques for samples prepared with 6%, 15%, and 20% biomass pigments.
   - **Drying time** was measured on standard paper substrates using pens and stamps, with rapid drying observed due to ethanol inclusion.

Other tests which involve toxicity analysis and deinking capacity are yet to be conducted.

Result and Conclusion:

In an era marked by industrialization and increasing environmental pollution, this project explores a sustainable alternative to conventional petroleum-based inks through the development of a soy oil– and algae-based bio-ink. Soybeans were processed and oil was extracted using Soxhlet extraction. Ethanol yielded minimal oil (1 mL from 20 g), whereas hexane proved more efficient with 30–35% oil yield and higher solvent recovery (78–82%).

Phycocyanin pigment was extracted from *Spirulina platensis* using phosphate buffer and NaCl, with phosphate buffer yielding higher pigment concentrations (up to 3.245 mg/mL). Increasing biomass concentration to 20% from 6% and 15% enhanced pigment color intensity. Crude pigment extract was directly used in the ink to avoid separation issues seen with lyophilized pigment.

Spectrophotometric analysis confirmed pigment identity, with characteristic peaks at 260 nm and 620 nm confirming the presence of protein family -phycobiliprotein and blue pigment respectively. The formulated ink showed improved drying time (2–3 seconds with ethanol addition) and densities ranging from 0.938–1.054 g/mL . Toxicity tests and de-inking tests are yet to be performed.

This study underscores the environmental and industrial potential of bio-sustainable inks, aligning with global efforts for greener, cleaner technologies.

Project Outcome & Industry Relevance:

This project demonstrates the successful development of a bio-sustainable ink formulated using soybean oil and phycocyanin pigment from *Spirulina sp.*, highlighting its practical potential as an eco-friendly alternative to conventional petrochemical-based inks. The ink is a carbon negative formula due to the inclusion of a renowned carbon consumer- algae . It is also biodegradable, non-toxic, and derived entirely from renewable sources. In the future , when the ink is made heat stable by using stabilizers like renewable sugar molecules (glucose etc) making it particularly relevant to industries such as packaging, publishing, and textile printing, where environmental compliance and consumer demand for green products are rapidly growing. The reduction of VOC emissions and the use of natural additives align with sustainability goals and government policies on environmental regulation. Additionally, this project supports the principles of a circular economy by utilizing agricultural and algal by-products. The formulation can be scaled up for industrial applications with minor modifications since it majorly uses processes like mechanical agitation for pigment extraction, distillation, roasting , making it highly viable for real-world implementation in sectors prioritizing sustainability.

Working Model vs. Simulation/Study:

This project involved the development of a physical working model in the form of a functional, bio-sustainable ink synthesized from soybean oil and Spirulina-derived phycocyanin. The research was conducted through extensive laboratory experimentation, including extraction, formulation, and characterization of the ink. All procedures were practically implemented using wet-lab techniques, and the final product was subjected to physical property testing, biodegradability assessments, and toxicity evaluations. As such, the project was not purely theoretical or simulation-based, but rather grounded in hands-on experimental work leading to a tangible, testable output.

Project Outcomes and Learnings:

Key outcomes of the project include the successful extraction and purification of soybean oil and phycocyanin pigment, the formulation of a stable natural ink with satisfactory drying time of 2-3 seconds, which is comparable to that of commercially available, carbon-based liquid inks. Through the process, our team gained hands-on experience in biochemical extraction techniques, formulation science, and characterization protocols. We also learned about the challenges of phase immiscibility and how formulation chemistry can be adjusted to overcome them. Additionally, the project enhanced our understanding of sustainable materials and provided valuable insights into developing eco-conscious products with real industrial applicability.

Future Scope:

The project presents significant potential for further development in sustainable ink technology. One promising direction involves intensifying the pigment color by incorporating natural metabolites such as tannins, which enhance color vibrancy and stability. To improve heat stability, sugars like glucose can be added as carriers. These sugars may be sustainably sourced from agricultural waste using enzymatic, alkaline, or acid hydrolysis methods, promoting a circular bio-economy.

Another innovative extension is the formulation of water-in-oil emulsion-based inks, resulting in thicker, paste-like inks suitable for 3D bio-printing applications. This could open pathways for use in biomedical printing and tissue engineering.

Further research can focus on scaling up production, improving storage stability, and enhancing compatibility with commercial printers. Investigating the long-term biodegradability and in vivo toxicity on mammalian cells would also support broader safety validation.

Overall, the project provides a strong foundation for eco-friendly ink innovations, aligning with global efforts to reduce environmental impact and develop carbon-negative, sustainable alternatives to synthetic inks.