



# Arthrospira Cell Residues for Lactic Acid Fermentation as Bioproducts From Waste Utilization

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*Arthrospira (Spirulina) platensis* is a freshwater cyanobacterium that is commercially produced as a food source on a global scale and considered safe for human consumption. After C-phycoerythrin (C-PC) extraction, the waste cell residue (WCR) is composed of nutrients with 30% protein content. Here, the potential of WCR as a nitrogen source for *Bacillus coagulans* cultivation and bioproducts was evaluated. Nitrogen substitute from WCR under different conditions of 20–100 g L<sup>-1</sup> was performed. *B. coagulans* cultivation was achieved with maximal viable cells at 7.6–9.5 log CFU mL<sup>-1</sup>. Increasing WCR gave decreasing lactate production. The highest lactate production at 27 g L<sup>-1</sup> was achieved from WCR 20 g L<sup>-1</sup>, highlighting the potential use of *A. platensis* waste biomass residue as a nitrogen source for the growth of lactic acid bacteria and zero-waste biotechnology methodology. The utilization of renewable resources is a crucial step toward developing a sustainable industrial society.

**Keywords:** *Arthrospira*, waste cell residue, *Bacillus coagulans*, bioproduct, zero-waste

## INTRODUCTION

*Arthrospira (Spirulina) platensis* cyanobacteria have attracted increased commercial interest due to their high protein content, essential amino acids, fatty acids, and pigments (De la Jara et al., 2018). The rich source of biochemical composition has become an interest in the food and health industry, and *Arthrospira* has shown value from the medical point of view (Furmaniak et al., 2017). Microalgal biomass is a valuable natural source of bioactive compounds that can be used in a variety of applications including the food sector (Ferreira et al., 2021), pharmaceuticals and nutraceuticals (Jha et al., 2017; Mehariya et al., 2021), therapeutic potentials (Khavari et al., 2021), cosmetics (Yarkent et al., 2020), biodiesel and biogas (González-González et al., 2018), and wastewater bioremediation (De Souza et al., 2022). *Arthrospira*, also known as *Spirulina*, is a well-known microalgal strain that has been globally cultured on a large commercial scale (Mostafa and El-Gendy, 2017). *A. platensis* biomass showed high potential as a source of various value-added product applications including bioethanol from carbohydrate and feed supplements because of its high protein and fatty acid contents (Raja et al., 2016). Previous studies demonstrated bioethanol production from *A. platensis*

**Abbreviations:**  $X_m$ , maximal cell growth (Log CFU mL<sup>-1</sup>);  $C_p$ , maximal lactate concentration (g L<sup>-1</sup>);  $Q_p$ , volumetric productivity of lactate production (g L<sup>-1</sup> h<sup>-1</sup>);  $Y_{p/s}$ , lactate yield coefficient (g g<sup>-1</sup>); *Yield*, conversion of glucose into lactate with a theoretical yield of 1.0 g lactate g<sup>-1</sup> glucose.

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biomass and biomethane production from waste cell residue (WCR) (Rempel et al., 2019). Chng et al. (2016) studied the production of bioethanol using lipid-extracted biomass from *Scenedesmus dimorphus* microalgae. Bioproducts from microalgae or waste cell residues are directly produced by fermentation or hydrolysis of the fraction before fermentation. Recent studies have shown the effectiveness of *Arthrospira* in the prevention of diabetes as an antiviral, immune-stimulator, and anticancer agent, with digestive improvement as well as growth of *Lactobacilli* (Christaki et al., 2011; Nicoletti, 2016). Several products from microalgal and cyanobacterial biorefineries with minimal waste outputs have been approved for the powerful valorization of biomass (Prabha et al., 2022).

Lactic acid fermentation as a food preservative method significantly improves the safety, shelf life, and nutritional properties of foods (De Marco Castro et al., 2019; Thompson et al., 2020) and is utilized in around 70% of food industry products (Eş et al., 2018). Fermentative biorefineries have wide-ranging product potential including fermented vegetables, fermented milk, meat industries, bio-preservatives, probiotics chemicals, biofuels, and pharmaceuticals (Raj et al., 2022).

Moreover, lactic acid fermentation has the potential for green production, biodegradation, and biocompatible polylactic acid (PLA) substitution for petroleum-based plastic (Nduko and Taguchi, 2021). Lactic acid fermentation can produce three forms of lactic acid, namely, L-lactate, D-lactate, or both D- and L-lactates depending on the microorganism used (Prasirtsak et al., 2019). *Bacillus coagulans* is a lactic acid-producing, spore-forming, catalase-positive bacterium with an optimal growth temperature of 35–50°C. These bacteria can utilize glucose, sucrose, lactose, and mannitol to produce L-lactic acid (Cao et al., 2020). Moreover, *B. coagulans* has also been accorded the Generally Recognized as Safe (GRAS) status by the US Food and Drug Administration (FDA) (Endres et al., 2009; Hazards, 2013). *B. coagulans* has significant benefits to the host immune system (Cao et al., 2020). Currently, *B. coagulans* is used as a functional food in chocolate, ice cream, and pasta. The spore-forming nature of *B. coagulans* is comparable to other probiotic bacterial strains, with high stability in functional food products (Majeed et al., 2016). Previous studies reported that lactic acid bacteria (LAB) have the ability to degrade the cell walls of plants and cyanobacteria through hydrolysis, resulting in the conversion of complex organic compounds such as polysaccharides, lipids, and proteins within the cells into smaller molecules with enhanced properties (De Marco Castro et al., 2019).

Algae and cyanobacteria have high nutritional values as suitable substrates for fermented food production. Fermented products from seaweed, microalgae, and *Arthrospira* were obtained as plant-derived substrates using various fermentation processes of lactic acid bacteria, yeast, or mixed cultures of microorganisms. These products are highly desired in the market with bioproduct applications (Gupta and Abu-Ghannam, 2011; Uchida and Miyoshi, 2013; Niccolai et al., 2019). Most previous reports studied microalgae as feedstock for lactic acid production using lactobacilli as the lactic acid bacteria. The hydrolysate of microalgae *Chlorella vulgaris* ESP-31

was used as feedstock for lactate production from *Lactobacillus plantarum* 23 (Chen et al., 2020) and *L. brevis* (Ścieszka and Klewicka, 2020), while whole freeze-dried *Arthrospira* biomass was used as the substrate for lactate production by *L. plantarum* ATCC 8014 (Niccolai et al., 2019; Niccolai et al., 2020) and wet *Arthrospira* biomass was used as the substrate in fermentation by *L. plantarum* ATCC 8014 for nutraceutical properties (De Marco Castro et al., 2019). However, large-scale lactate production from microorganisms involves the high cost of fermentation media as one of the most critical bottlenecks in industrial lactate production (Cubas-Cano et al., 2018). Lactic acid bacteria (LAB) require complex nutrition that consists of high carbon sources, amino acids, vitamins, and nucleotides (Mokoena, 2017). Nitrogen, vitamins, and other nitrogenous growth-stimulating elements are abundant in yeast extract and peptone (Abbasiliasi et al., 2017). Organic nitrogen sources such as yeast extract, peptone, and other proteinous substrates must be added to LAB culture media, increasing the cost of medium formulation. As a result, it is important to discover low-cost alternatives for these expensive ingredients. *Bacillus* strains can grow and produce L-lactic acid under minimal nutritional requirements (Poudel et al., 2016).

Few studies have focused on producing lactate from waste cells of microalgal biomass in a biorefinery as a zero-waste process, while lactate production from *Bacillus coagulans* has never been reported. Waste microalgae and cyanobacteria can be used as an alternative nitrogen source for lactic acid production, representing bioproducts as a renewable resource with reduced production cost. Therefore, this study was conducted to investigate the potential of novelty waste *A. platensis* cell residues after C-phycoerythrin extraction in a culture medium by *B. coagulans* ATCC 7050 for lactate production as a renewable resource.

## MATERIALS AND METHODS

### Materials

Analytical grade Zarrouk medium composed of sodium bicarbonate, sodium nitrate, dipotassium phosphate, potassium sulfate, sodium chloride, magnesium sulfate, calcium chloride, iron sulfate, ethylenediaminetetraacetic acid, and other reagents was purchased from Ajax Finechem Pty Ltd. (Auckland, New Zealand). GYP medium composed of glucose monohydrate, granulated yeast extract, peptone from meat, and other reagents was purchased from EMD Millipore Corporation (Burlington, United States). *Bacillus coagulans* ATCC 7050 was purchased from the American Type Culture Collection (ATCC, United States).

### Microalgae Cultivation and Preparation

#### Microalgae Preparation

*Arthrospira (Spirulina) platensis* IFRPD 1182 was obtained from the Algae Laboratory at the Institute of Food Research and Product Development, Kasetsart University, Thailand. *A. platensis* was maintained and cultivated in a modified Zarrouk medium (Pan-utai et al., 2020) in photobioreactors and then

scaled up to a 200-L outdoor open raceway pond. The biomass of *A. platensis* was grown to log phase and then harvested by nylon membrane filtration. The harvested cells were cleaned with fresh water to remove any residual culture medium. *A. platensis* biomass preparation was dried in a hot air oven (Thermo Scientific, Germany) at 60°C for 6 h and then milled to 0.5 mm particle size.

### Waste Cell Residue Preparation

C-phycoerythrin (C-PC) was extracted from *A. platensis* biomass under optimal conditions (Pan-utai et al., 2018) of biomass solvent ratio 1:15 (w/v) with 10 mM phosphate buffer at pH 7.0 and incubated under a controlled extraction temperature of 25°C for 24 h. The C-phycoerythrin (C-PC) supernatant was then separated by centrifugation at 10,000  $\times$  g for 30 min (Model 6000, High-Speed Refrigerated Centrifuge, KUBOTA, Japan) at 25°C. After C-PC extraction, the cell pellet remained as the waste cell residue (WCR). This residue was dried in a hot air oven at 60°C for 6 h and then milled to 0.5 mm particle size for further analysis as a substrate for lactic acid fermentation.

### Biochemical Composition

The prepared *A. platensis* biomass and waste cell residue (WCR) were analyzed to determine their biochemical compositions following NREL methods. Moisture content was determined by oven-drying at 105°C to constant weight (Sluiter et al., 2008a). Ash content was determined by ignition of the dried samples in an electric furnace at 550°C (Sluiter et al., 2008b). Protein content was determined by the Kjeldahl method (Hames et al., 2008), and lipid content was determined using a modified Bligh and Dyer method (Pan-utai et al., 2019). Briefly, the samples were suspended in distilled water, methanol, and chloroform at a ratio of 0.8:2.0:1.0 and mixed well. The mixture was ultrasonically homogenized for 15 min and then separated by centrifuging at 6,153  $\times$  g for 15 min. The lipid phase was collected, and the cell debris was extracted until the cells had no color. The lipid extract was filtered to remove contaminated cell debris and dried to constant weight at 80°C. After extraction of C-PC, the supernatant was collected, and the absorbance was determined at 615 and 652 nm using a UV-vis spectrophotometer (SP-8001, UV/Vis Spectrophotometer, Metertech, Taiwan) (Pan-utai and Iamtham, 2019). C-phycoerythrin content (C-PC) is calculated by Eq. 1 as follows:

$$C - PC \text{ (mg mL}^{-1}\text{)} = \frac{OD_{615} - 0.474OD_{652}}{5.34} \quad (1)$$

### Pigment Determination

Chlorophyll was extracted from the samples with methanol and stored at 4°C for 24 h. The optical density of the supernatant was measured at 662 and 645 nm. The chlorophyll content is calculated from the combination of chlorophyll a and b using the following Eqs. 2, 3 (Pan-utai et al., 2021):

$$\text{Chlorophyll a (mg mL}^{-1}\text{)} = 11.75OD_{662} - 2.35OD_{645} \quad (2)$$

$$\text{Chlorophyll b (mg mL}^{-1}\text{)} = 18.61OD_{645} - 3.96OD_{662} \quad (3)$$

### Glucose Analysis

Glucose in waste cell residues was determined by analysis of the carbohydrate in the biomass following Yuan et al. (2016). WCR *A. platensis* biomass was hydrolyzed by 72% (w/w) sulfuric acid at 30°C for 1 h. The acid concentration of the samples was then adjusted to 4% (w/w), and the samples were sterilized at 121°C for 1 h. The glucose concentration in the supernatant hydrolysate was determined on a Hewlett-Packard HP 1100 Series HPLC system with a refractive index detector (Agilent Technologies, United States), using a cross-linked resin hydrogen ionic 300  $\times$  7.8 mm Aminex HPX-87H (9  $\mu$ m) column (Bio-Rad). The column oven was set at 50°C, and the mobile phase was 5 mM sulfuric acid. The flow rate was set at 0.5 ml min<sup>-1</sup> using a refractive index detector at 40°C. Glucose contents in the samples are calculated according to Eq. 4:

$$\text{Glucose content (\%)} = \frac{\text{Glucose produced (g)}}{\text{Dry weight of sample}} \times 100. \quad (4)$$

## FERMENTATION

### Inoculum Preparation

Lactate production was performed by *B. coagulans* ATCC 7050 maintained in GYP medium and 10% skim milk and stored at -20°C. The GYP medium had the following composition (g L<sup>-1</sup>): glucose 10 g, yeast extract 5 g, peptone 5 g, KH<sub>2</sub>PO<sub>4</sub> 0.25 g, K<sub>2</sub>HPO<sub>4</sub> 0.25 g, and salt solution 10 ml consisting of (L<sup>-1</sup>) MgSO<sub>4</sub>·7H<sub>2</sub>O 40 g, MnSO<sub>4</sub>·5H<sub>2</sub>O 2 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 2 g, and NaCl 2 g. The pH value of the GYP medium was adjusted to 6.8 (Thitiprasert et al., 2017). Primary inoculum preparation of *B. coagulans* ATCC 7050 was cultured in GY medium consisting of (g L<sup>-1</sup>): glucose 10 g, yeast extract 15 g, NH<sub>4</sub>Cl 4 g, KH<sub>2</sub>PO<sub>4</sub> 0.5 g, K<sub>2</sub>HPO<sub>4</sub> 0.5 g, and salt solution 20 ml. The pH value of the GY medium was adjusted to 6.8 (Jaiuae et al., 2021). The inoculum culture was placed in an incubator shaker at 200 rpm, with temperature controlled at 37°C for 18 h. Then, 10% (v v<sup>-1</sup>) of *B. coagulans* ATCC 7050 cells were transferred to secondary inoculum preparation, cultured at 37°C, 200 rpm for 6 h, and used as the starter for *B. coagulans* cultivation.

### Lactate Production by *B. coagulans*

WCR was used as the supplemented feedstock for lactate production under different concentrations of 20, 40, 60, 80, and 100 g L<sup>-1</sup> in the culture medium. The control experiment was conducted without WCR. The medium formulation was modified by the culture medium corresponding to lactate fermentation from 120 g L<sup>-1</sup> of initial glucose concentration (Tolieng et al., 2018). WCR was used to replace yeast extract in the culture medium, consisting of (g L<sup>-1</sup>): glucose 120 g, NH<sub>4</sub>Cl 2 g, KH<sub>2</sub>PO<sub>4</sub> 0.25 g, K<sub>2</sub>HPO<sub>4</sub> 0.25 g, and salt solution 10 ml. Then, the culture medium was sterilized at 121°C for 15 min. *B. coagulans* ATCC 7050 inoculum at 10% (v v<sup>-1</sup>) was transferred into the culture medium for growth and lactate

production. The cultivation was performed at a working volume of 50 ml medium in a 250-ml Erlenmeyer flask. The culture was incubated at 37°C in a shaker at 200 rpm. Fermented samples were collected at the start of fermentation and every 12 h for viable cell growth, glucose, and lactic acid determination. The pH was measured using a pH meter (Lab 850, Schott, Germany).

### Viable Cell Determination

*B. coagulans* ATCC 7050 was collected during the fermentation process for viable cell determination. Colony-forming units (CFU) were counted in 1 ml sample collected from the fermentation broth. The fermentation broth was diluted to an appropriate multiple with sterile water, and then 100  $\mu$ L sample was spread on a nutrient agar (NA) plate and cultured at 37°C for 48 h.

### Glucose and Lactate Determination

Fermentation samples were collected and centrifuged at 6,153  $\times$ g for 20 min, with the supernatant stored at -20°C until analysis. Glucose, lactate, acetate, and ethanol were analyzed using high-performance liquid chromatography (HPLC). Cell-free fermentation broth samples were filtered through a nylon membrane, while the remaining glucose and lactate products were determined using a Hewlett-Packard HP 1100 Series HPLC system with a reflective index detector (Agilent Technologies, United States) and a 300 mm  $\times$  7.8 mm Aminex HPX-87H organic acid column (9  $\mu$ m particle size and 8% cross-linkage) (Bio-Rad). The column was maintained at 50°C in a column oven. The sample was injected and eluted with 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.5 ml min<sup>-1</sup>. Glucose anhydrous (CAS-No. 108337, Merck, Germany), L-lactic acid (CAS-No. 46937, Sigma-Aldrich, Singapore), acetic acid (Glacial, AR grade, Ajax Finechem Pty Ltd., Auckland, New Zealand), and absolute ethanol (99.5%, GR grade, DUKSAN, South Korea) were used as external standards to calculate the concentration from the peak area.

### Kinetic Parameters

Viable cells of *B. coagulans* ATCC 7050 were assessed for growth and lactate production, and the kinetic parameters were calculated. The maximal value of viable cell growth was  $X_m$ . Lactate productivity ( $Q_p$ ) was calculated as the ratio of variation in lactate concentration ( $P_m - P_0$ ) to fermentation time, as shown in Eq. 5:

$$Q_p (g L^{-1} h^{-1}) = \frac{P_m - P_0}{dt} \quad (5)$$

The lactate yield coefficient is calculated using Eq. 6:

$$Y_{P/S} (g g^{-1}) = \frac{\Delta P}{\Delta S} \quad (6)$$

where  $Y_{P/S}$  is the lactate yield coefficient in  $g g^{-1}$  unit. Abbreviations of  $\Delta P$  and  $\Delta S$  represent differences in lactate and glucose production, respectively.

## RESULTS

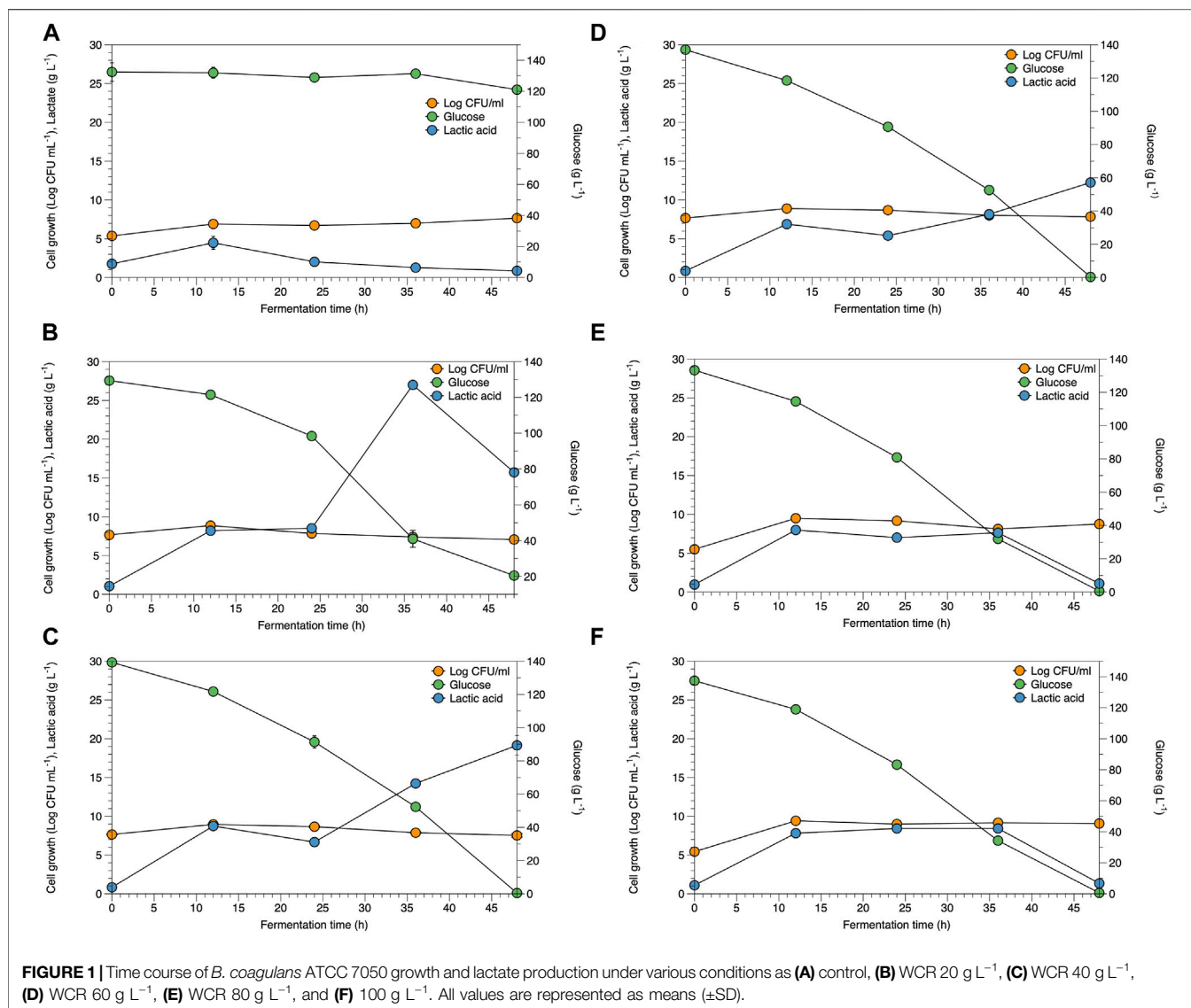
### Biochemical Composition of the Microalgae

*A. platensis* IFRPD 1182 was used as the initial substrate, which was prepared as both microalgal biomass and waste cell residue (WCR) from C-PC extraction and also used as the substrate for fermentation as of renewable resource. Biochemical compositions of *Arthrospira platensis* IFRPD 1182 microalgal biomass and WCR are shown in **Supplementary Table S1**. The initial *A. platensis* biomass had the highest protein and C-PC contents. C-PC is a blue pigment composed of proteins. After C-PC extraction, the C-PC remaining contained 8.33% ( $mg g^{-1}$ ) as around 12.6% of C-PC in the initial biomass. However, 30% (DW) protein content and more than 63% of the initial biomass, including other compositions, remained in the WCR. Therefore, the potential of the remaining WCR nutrition was studied as an alternative nitrogen source for lactate production.

### Potential of *B. coagulans* ATCC 7050 Cultivation

The potential of WCR substitution for nitrogen sources in the fermentation of lactic acid bacteria was studied as a low-cost medium and renewable resource. Lactate fermentation by *B. coagulans* ATCC 7050 with different WCR concentrations at 20–100  $g L^{-1}$  substituted for yeast extract as the alternative nitrogen source was studied in the culture broth. Parameters of cell growth, glucose consumed, and lactate produced are shown in **Figure 1**. Various WCR concentrations were studied to substitute nitrogen sources in the fermentation medium of *B. coagulans* ATCC 7050. The fermentation medium without WCR and yeast extract was used as the control (**Figure 1A**). *B. coagulans* ATCC 7050 cells grown at different WCR concentrations and constant glucose of 120  $g L^{-1}$  reached exponential values within 12 h of fermentation, while glucose concentration slightly reduced at 48 h of fermentation. A maximal lactate concentration was obtained after 12 h of fermentation at 4.5  $g L^{-1}$ . The results from the control experiments without a nitrogen source in the fermentation medium showed that *B. coagulans* was not appropriate for cell growth and lactate production. Fermentation in all experiments produced by-products, shown in **Figure 2**. At longer fermentation time, acetate and ethanol production increased with also large amounts of by-products.

Viable *B. coagulans* ATCC 7050 growth at different WCR concentrations during fermentation are shown in **Figures 1B–F**. The results indicated that higher WCR concentration led to higher viable cell growth, while glucose as the carbon source in the culture media was almost completely consumed by *B. coagulans* at the end of fermentation. Lactate production at different treatments increased with cell growth and fermentation time. **Supplementary Table S2** shows the pH values during fermentation at various treatments. *B. coagulans* ATCC 7050 growth and lactate production led to an increase in acidity from 6 to 4.6. Maximal *B. coagulans* ATCC 7050 viable growth at various WCR concentrations without a nitrogen source



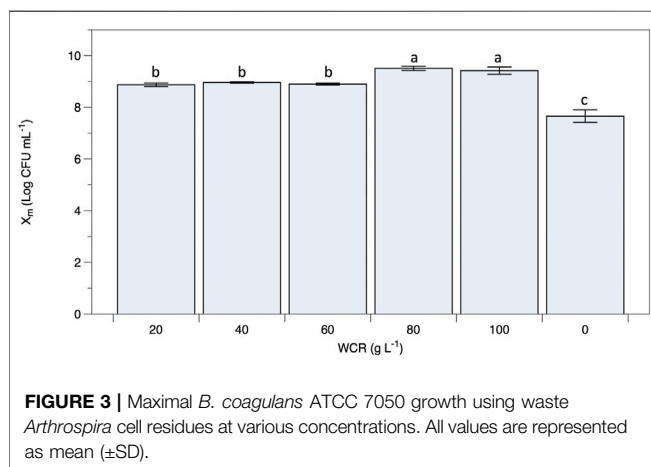
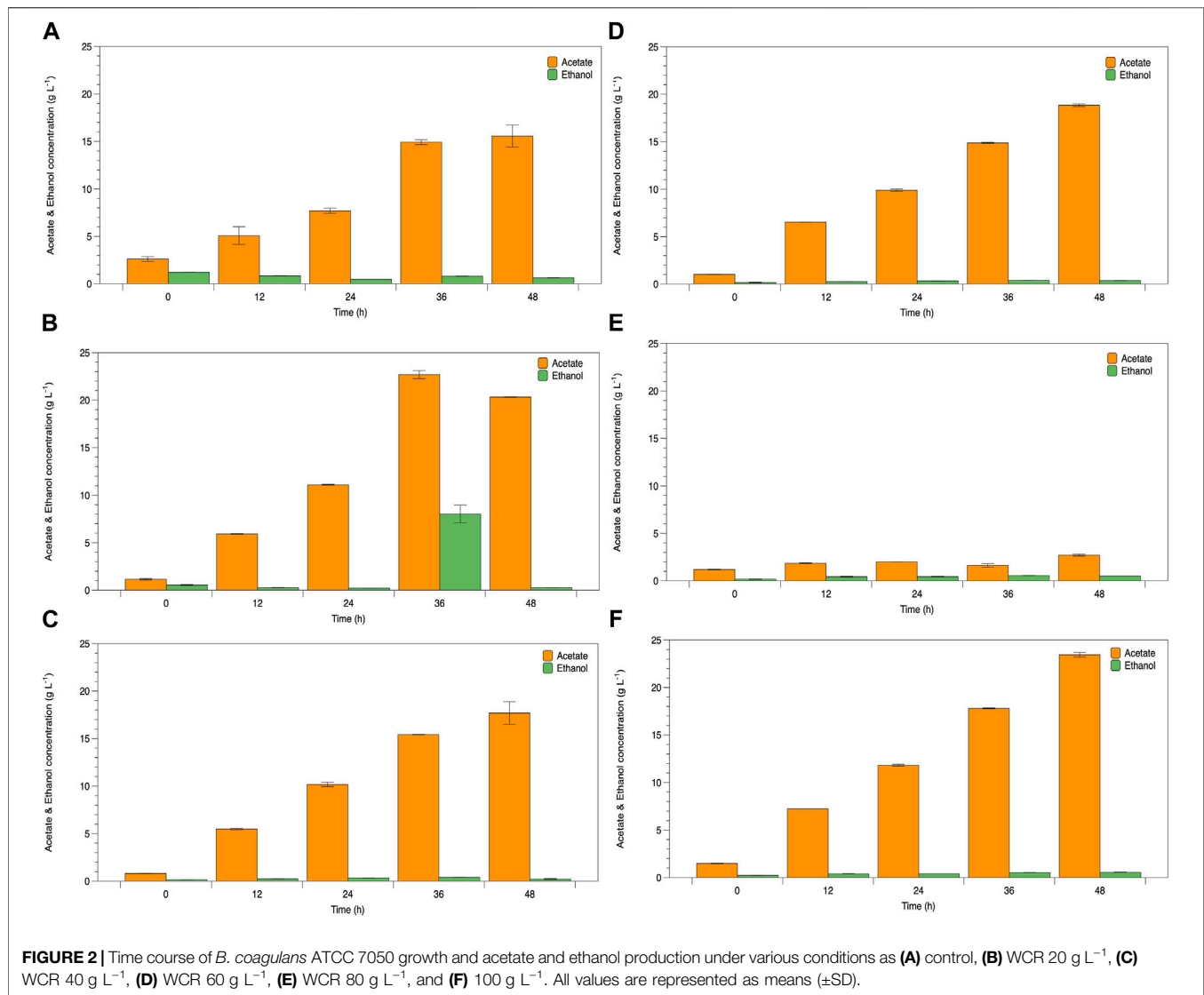
is shown in **Figure 2**. *B. coagulans* ATCC 7050 grew and increased during fermentation. Maximal cell growth at various WCR concentrations ranged from 7.6 to 9.5 log CFU mL<sup>-1</sup> (**Figure 3**). Increasing WCR concentration in the fermentation media gave viable cell growth, whereas viable *B. coagulans* ATCC 7050 decreased in the treatment without WCR and other nitrogen sources (control experiment).

Parameters of *B. coagulans* ATCC 7050 fermentation at different WCR conditions for potential lactate production are shown in **Supplementary Table S3**. Maximal lactate concentration ( $C_P$ ) under various WCR concentrations ranged from 8 to 27 g L<sup>-1</sup>, with the highest recorded at 20 g L<sup>-1</sup>. Higher WCR concentration in the fermentation broth decreased lactate production. Lactate productivity ( $Q_P$ ) ranged from 0.23 to 0.75 g L<sup>-1</sup> h<sup>-1</sup> under different WCR concentrations in the fermentation process. Maximal lactate concentration and productivity were recorded at 20 g L<sup>-1</sup> WCR and not significantly different from 80 g L<sup>-1</sup>. The maximal lactate yield coefficient ( $Y_{P/S}$ ) and

theoretical yield of lactate ( $Yield$ ) were obtained from *B. coagulans* ATCC 7050 fermentation using 20 and 80 g L<sup>-1</sup> WCR concentration, with a nonsignificant difference compared to the control. The optimal condition for *B. coagulans* ATCC 7050 fermentation, giving a maximal yield of lactate, was 80 g L<sup>-1</sup> WCR.

## DISCUSSION

*Arthrospira* is well known as a rich protein source with high nutritional value. The nutritional composition of WCR showed useful protein, carbohydrate, and lipid content, with C-phycoyanin and chlorophyll contents of  $8.33 \pm 0.54$  and  $13.43 \pm 0.17$  mg g<sup>-1</sup> DW, respectively. Previous reports recorded that wet biomass of *Arthrospira* gave enhanced nutraceutical properties by fermentation with *Lactobacillus plantarum* (De Marco Castro et al., 2019). Microalgal biomass



of *A. platensis* was used as feedstock for biogas production (Dębowski et al., 2020), while *A. platensis* solid-state fermentation with LAB was performed for aromatic profile evaluation (Martelli et al., 2021). Generally, simple sugars such as glucose and sucrose are used as carbon sources for lactate fermentation (Olszewska-Widdrat et al., 2020). Most previous reports focused on *Arthrospira* with complete nutritional enrichment of microalgae. Carbohydrates from microalgae can be converted to fermentable sugars by hydrolytic processes. However, the fermentation requires large amounts of carbohydrate at up to more than 70% DW of the microalgal biomass, limiting control in large-scale cultivation of carbohydrate-enriched *Arthrospira* (Liu et al., 2019). Our results showed glucose content from WCR biomass using acid hydrolysis followed by HPLC determination as  $35.50 \pm 0.13\%$

DW. Various WCR concentrations were used as feedstock addition fermentation at 20, 40, 60, 80, and 100 g L<sup>-1</sup>, with hydrolyzed glucose contents of 7.10, 14.20, 21.30, 28.40, and 35.50 g L<sup>-1</sup>, respectively. WCR was sterilized at 121°C for 15 min without pretreatment with acid hydrolysis and performed the same as culture broth giving glucose content at only 0.3% DW. Thus, the addition of WCR substitute in culture media at 0.06–0.30 g L<sup>-1</sup> gave reduced glucose with increasing media formulation. Our results showed mainly protein content in the biochemical composition of nutrition values from WCR. Yeast extract is a nutrient source of vitamins and amino acids for microorganism growth, with high cost at approximately 38% of the total medium cost in lactate production (Meng et al., 2012; Ma et al., 2014). Therefore, WCR shows significant potential as a source of nitrogen feedstock to produce bioproducts. In our study, fermentation of WCR as the nitrogen source was evaluated for *B. coagulans* growth and lactate production.

*B. coagulans* cultivation from WCR addition in culture media at different loadings showed variable cell growth and lactate production. Increasing WCR gave higher maximal viable cell growth because the biomolecules of WCR containing amino acids, vitamins, and proteins supported cell growth. The control experiments without WCR and nitrogen source addition found that *B. coagulans* could use glucose as the carbon source. However, cell growth limitation was stimulated by other nutrients necessary for complete cell growth. Oh et al. (2003) confirmed that yeast extract was the most effective nitrogen source for cell growth and lactate production. Amino acids are important molecules that serve as the fundamental components or building blocks of proteins (Moini, 2019). They play a significant role in facilitating microbial cell growth (Idrees et al., 2020). Moreover, amino acids including glycine, serine, and tyrosine in yeast extract play a central role in promoting cell growth (Li et al., 2011). The protein content of *Arthrospira* is high and contains all the essential amino acids with good digestibility (Bernartts et al., 2019; Niccolai et al., 2019). Khandual et al. (2021) found large amounts of each amino acid in the total biomass after C-phycocyanin extraction. Therefore, WCR addition in *B. coagulans* culture media stimulated microbial cell growth.

Viable cell growth increased when WCR feedstock loading increased, while lactate production increased with fermentation time. WCR loading in the culture medium stimulated cell growth and lactate production, as confirmed by the control experiments. The results showed glucose consumption to produce lactate. Moreover, during *B. coagulans* ATCC 7050 fermentation supplemented with WCR, various LAB fermentation metabolites such as lactate, acetate, ethanol, aroma compounds, and enzymes were produced (Raj et al., 2022). *B. coagulans* produces homofermentative lactic acid under anaerobic conditions (Okada et al., 1979). However, this homofermentation reaction also produces by-products including acetate kinase and alcohol dehydrogenase in the acetate and ethanol conversion process. Our results showed that both lactate and by-products were produced under unsuitable fermentation conditions. Su and Xu (2014) also determined ethanol and acetate as the primary fermentation

products under aerobic conditions. The acetate kinase-encoding gene present in the *B. coagulans* genome prefers an energy-generating pathway, with acetic acid synthesis to metabolize accumulated NADH following the loss of the lactate dehydrogenase function (Sun et al., 2016). These reasons support our results. Moreover, lactate production by glucose fermentation of *B. coagulans* was inhibited by glucose concentrations higher than 100 g L<sup>-1</sup> (Michelson et al., 2006), leading to decreased growth rate and underfermentation (Glaser and Venus, 2018). Lactic acid-producing bacteria are metabolized by the phosphogluconase pathway that produces various co-products such as acetate, CO<sub>2</sub>, and ethanol, with low lactate yield as the end product of fermentation (Bintsis, 2018; Abedi and Hashemi, 2020). Pyruvate molecules can be reduced to lactate, with acetate to ethanol and CO<sub>2</sub> (Mendes Ferreira and Mendes-Faia, 2020). These were used as a carbon source for lactate and bioproducts in this study. Therefore, the relationship between the quantities of acetate and ethanol that decreased the theoretical yield to 0.50 g g<sup>-1</sup> depends on the capacity of bacteria to reoxidize NADH created in the initial procedure, as well as its energy demands (Yu et al., 2020).

Kinetic parameters of *B. coagulans* ATCC 7050 cultivation using WCR substituted by yeast extract were calculated in terms of fermentation time for maximal lactate production at 12 h. WCR addition achieved higher lactate production, with increased nutrients in the culture media associated with cell growth. Maximal lactate yield was achieved at WCR addition of 20 g L<sup>-1</sup>, indicating sufficient nitrogen and other nutrients for direct utilization of cell growth. When WCR loading increased, the pigments of C-PC and chlorophyll also increased, promoting the nutraceutical properties of *Arthrospira* fermentation (De Marco Castro et al., 2019). However, higher pigments and total phenolic compounds may inhibit bacteria cultivation (Takó et al., 2020) as well as have a positive effect on microorganisms (Adebo and Gabriela Medina-Meza, 2020). *Arthrospira* biomass can also serve as a nutrient source during the fermentation process (Luiza Astolfi et al., 2020). Moreover, *B. coagulans* is catalase-positive and produces energy from glucose via glycolysis, the citric acid cycle (TCA), and the electron transport chain, resulting in high growth rates. Lactate was produced with high productivity at higher cell growth (Thitiprasert et al., 2017). Therefore, WCR was utilized as a renewable resource to replace expensive yeast extract for lactate production by *B. coagulans*.

**Supplementary Table S4** compares previous fermentation studies of bioproducts produced from various substances. Lactate production from lyophilized biomass cyanobacterium *Arthrospira platensis* F&M-C256 by *Lactobacillus plantarum* ATCC 8014 reached 3.7 g L<sup>-1</sup> lactate concentration (Niccolai et al., 2019). *A. platensis* F&M-C256 was also successfully evaluated in a vegetal soybean drink and in water by probiotic *L. plantarum* ATCC 8014 at 1.7 g L<sup>-1</sup> (Niccolai et al., 2020). Our results showed the highest lactate production due to different bacterial strains and substrate consumed. Using WCR as the nitrogen source reduced the cost of the culture medium with direct utilization without pretreatment. Werlang et al. (2020) studied *A. platensis* as a raw material for D-lactic acid production via saccharification and fermentation enzymatic processes using the lactogenic *Escherichia coli* strain JU15, giving D-lactate at

25.5 g L<sup>-1</sup>, as an alternative option for renewable resources as the carbon source for bacterial cultivation. *B. coagulans* can produce L-lactate by fermentation generally in a synthetic medium. L-Lactate production by *B. coagulans* has been studied using different fermentation medium compositions and processes (Fan et al., 2016; Tolieng et al., 2018). Here, alternative nitrogen sources to replace yeast extract in the fermentation medium realized higher L-lactate concentrations. Other substrates as the fermentation medium and processes for lactate production were applied to cassava starch hydrolysates and sludge hydrolysates (Ma et al., 2014; Ooi and Wu, 2015). The complete GY culture medium obtained high lactate production (Tolieng et al., 2018). Our results gave lower lactate but still reduced medium cost and used WCR as a renewable resource for nutraceutical ingredients for health. Using *Arthrospira* biomass as the substrate for lactate production by the probiotic bacterium significantly increased essential amino acid contents and the bioactive profile through probiotic growth and pre- and probiotic bio-active substance production (De Marco Castro et al., 2019; Yu et al., 2020). Therefore, waste *Arthrospira* cell residue from C-PC extraction can act as an alternative substrate for probiotic *B. coagulans* cultivation and produce lactate as renewable bioproducts.

## CONCLUSION

Waste cell residue of *Arthrospira* IFRPD 1182 biomass from C-PC extraction showed feasibility as an alternative substrate for *B. coagulans* ATCC 7050 cultivation. WCR acted as a nitrogen source that replaced yeast extract in culture media, resulting in reduced production cost. *B. coagulans* ATCC 7050 cultivation gave maximal lactate yield at 20 g L<sup>-1</sup> WCR. However, the fermentation process should be further studied to give high lactate production in a shorter time, with *Arthrospira* saccharification and fermentation also investigated to enhance production efficiency. Our results demonstrated the feasibility of utilizing waste resources from the cyanobacterium *Arthrospira* to produce valuable products. WCR was proven as a significant

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alternative resource for *Bacillus coagulans* growth, bioproduct production, and also reducing waste in food systems.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

WP-u: conceptualization, formal analysis, writing—review and editing, and visualization. ST: conceptualization and validation. SP: validation.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenrg.2022.878597/full#supplementary-material>

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