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## *Abstract:*

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Article

# Choline-Based Ionic Liquids as Media for the Growth of *Saccharomyces cerevisiae*

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**Abstract:** Ionic liquids (ILs) have garnered great attention as alternative solvents in many biological reactions and applications. However, its unknown toxicity is in line with the challenges to use it for biological applications. In this study, three choline based Ionic Liquids—choline saccharinate (CS), choline dihydrogen phosphate (CDHP), and choline tryptophanate (CT) were assessed for their suitability on the growth of *Saccharomyces cerevisiae*. The ILs were incorporated into the growth media of *S. cerevisiae* (defined as synthetic media) to assess its potential as a substitute to conventional media. The compatibility of the synthetic media was evaluated based on the toxicity (EC<sub>50</sub>), growth curve, and glucose profile. The results showed that the incorporation of CDHP and CS did promote the growth of *S. cerevisiae* with a rapid glucose consumption rate. The growth of *S. cerevisiae* with the media composition of yeast extract, peptone, and CS showed improvement of 13%. We believe that these observations have implications in the biocompatibility studies of ILs to microorganisms.

**Keywords:** biotoxicity; choline; ionic liquids; *Saccharomyces cerevisiae*

## 1. Introduction

Growth media is a pivotal platform to ensure that microorganisms have all the necessary nutrients and components for an ideal growth, without any additional stresses. An ideal growth media should contain nutrients in the form of proteins or amino acids, an energy source in the form of carbohydrates, and some traces of essential minerals or salts. Sometimes, specialized media is needed to cater to specific growth parameters or to enhance the growth of specific microorganism. Synthetic media, usually a medley of various components has been widely applied in the cultivation of recombinant protein [1], algae [2,3], bacteria [4], yeast [5–7], and also mammalian cells [8]. The conventional media to cultivate *S. cerevisiae* is intended to provide the necessary components required for optimal growth of *S. cerevisiae* in a laboratory scale. The basic idea is to provide all the necessary nutrients such as carbon, nitrogen, vitamins and minerals. In this context, the most commonly used media is the Yeast Extract-Peptone-Dextrose (YPD) media. One of the components of the YPD media, yeast extract functions to provide the budding *S. cerevisiae* the necessary vitamins needed. Peptone is added as a source of carbon, nitrogen, vitamins and minerals, and dextrose on the other hand is added as a carbohydrate source. Apart from these, there are reports on the usage of synthetic media to specifically suit the growth needs of *S. cerevisiae*. These include synthetic minimal media [9–11] which contains nitrogen base without amino acids and also the synthetic complete media [12–14] which has a nitrogen

base as well as amino acid supplements. In recent years, the use of ILs as cultivation media for biocatalytic reactions and biotransformation has gained significant attention [15–17]. The unique property of ILs has earned them the name “green solvent” whereby their tunable properties can make them a tailored fit for a variety of functions [18,19]. These ILs are said to increase the growth rate of cells by either increasing the nutrient availability or, in some cases, metabolized as nutrients [15,17]. Choline based ILs have been found in the past to be excellent solvents/media for biocatalytic reactions [20]. Its ability to maintain cell conformation and catalytic activity is due to its low levels of toxicity [21]. Choline based salts (developed from physiological salts, sugars) represents a subset of biocompatible ILs which are often FDA approved [22]. Choline based ILs are significantly less toxic than pyridinium- and imidazolium-based ILs [23] and can improve the protein shelf life, retain structures and protein activity [7,24]. The choline based ILs are also reported to be almost completely biodegradable (93%) [25].

In unconventional surroundings, microorganisms tend to exhibit a dose-stimuli response (hormesis). The nature of the response depends highly on the agent that causes the stress, the intensity/duration of the stress, and also the physiological effect of the organism itself [26]. It is also interesting to note that in certain cases, hormesis activates certain defensive pathways that provides protection towards higher doses of the stress agent and sometimes towards other stressors as well. In other words, the organism begins to adapt towards the stressors and a certain defense pathway is activated to provide protection towards other toxic agents [27–30]. The hormetic response is said to be a better alternative to predict and extrapolate the toxicity of harmful chemicals than the conventional models [31].

The effect of ILs towards hormesis is to a varying degree. It was previously shown that certain IL contributes towards hormesis to a certain microorganism [31–34] and others do not [16,35]. Habitually, hormesis was applied to describe the effect of low dose ionizing radiation; however, over time, it has been adapted to describe a biphasic dose response. One such study has shown that emim[OAc] contributed to a remarkable hormesis effect towards *Clostridium sp.* and *P. putida* growth [33] in which growth was stimulated at  $2.5 \text{ g L}^{-1}$  of ILs but inhibited at  $>2.5 \text{ g L}^{-1}$  of ILs. The same scenario was observed with *Saccharomyces cerevisiae*, whereby Ouellet et al. [32] reported that emim[OAc] caused a severe inhibitory effect at a very low concentration; whereas Zhu et al. [31] reported that bmim[Cl] at high concentrations ( $>10^3 \text{ g L}^{-1}$ ) inhibited yeast growth. Due to all these contradicting findings towards the effect of ILs on a microorganism, it has thus garnered much interest in the scientific community to further study the extensive effect of ILs towards microorganisms. It is clear by this time that various ILs have different effects on different organisms.

The best model to study hormesis is to use a particular yeast species called *Saccharomyces cerevisiae*. This particular species of yeast is the most robust organism and has a simple lifecycle of mitosis and growth. In the past, this organism has been used as a model organism to conduct various studies ranging from cell cycle [36], to the study of neurodegenerative disease [37], although much is unknown on its toxicity [38]. The cultivation of yeast is relatively simple. They can be grown in either liquid medium or a solid agar plate. The media should contain a carbon source (glucose or dextrose), and salts that supply nitrogen, phosphorus, and trace metals.

The viability of *S. cerevisiae* after exposure to ILs has been extensively studied with regards to their toxicity [7,38], but their ability to grow and produce metabolites have not been studied [39]. A recent study conducted by Matsuda and team (2019) [7], studied the effects of two hydrophilic ILs: Choline lactate and 1-ethyl-3-methylimidazolium acetate mixed with yeast (*Schizosaccharomyces pombe*) culture medium. They found that the yeast cells maintained their bioactivity in the choline based ILs; whereas in the imidazolium based ILs, changes were observed in the protein secondary structure of the yeast cells. *S. cerevisiae* also displayed good biocompatibility with aqueous two-phase extraction systems [16]. Thus, further information on its biocompatibility to ILs would be vital. A comparison between the original medium (YPD) and the experimental medium for growth is presented in this study. It would be interesting to observe how choline based ILs affect the growth of *S. cerevisiae*. Therefore, this study examines the effect of 3 ILs, CS, CT, and CDHP as an ingredient in the generation

of yeast synthetic media in lieu to its growth profile. From our understanding, this is the first time ILs have been used wholly as a synthetic media for the growth of *S. cerevisiae*. We believe these preliminary results would be useful in providing an insight in utilizing ILs in media compositions for the growth of microorganisms.

## 2. Materials and Methods

### 2.1. Materials

All precursors and solvents were purchased from Sigma Aldrich, St. Louis, MO, USA and were used without further purification. Choline hydroxide solution (45 wt % in methanol) and Phosphoric acid ( $\geq 85\%$ ) were obtained from Sigma Aldrich, St. Louis, MO, USA. Activated charcoal (100 mesh,  $\geq 90\%$ ) was also purchased from Sigma-Aldrich, St. Louis, MO, USA. L-Tryptophan ( $\geq 99\%$ ), methanol and acetonitrile ( $\geq 99\%$ ), choline chloride, sodium saccharinate, absolute ethanol, cation-exchange resin (DOWEX 50W), aqueous HCl solution, anion-exchange resin (DOWEX 1\_2-400), aqueous sodium chloride solution, and silver nitrate were also purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals were purchased from Merck (Darmstadt, Germany) with analytical grade purity.

### 2.2. Methods

#### 2.2.1. Synthesis of ILs

All the ILs used in this experiment (CS, CDHP, and CT) were synthesized via similar metathesis reaction in which the detailed method has been adopted from previously reported work [40,41]. All three synthesized ILs were characterized by Nuclear Magnetic Resonance ( $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR spectrometer Bruker Avance II Plus 500 MHz) with deuterated water,  $\text{D}_2\text{O}$ , as the solvent.

#### 2.2.2. Microorganism and Culture Conditions

A stock culture of *S. cerevisiae* (#060M1510V, Sigma) was inoculated into a freshly prepared YPD medium containing 2% glucose, 2% peptone and 1% yeast extract. The culture was incubated overnight to achieve a significant growth of population. Growth curve was plotted by obtaining samples every hour. Samples were taken from the shake flask containing yeast and its media composition and UV absorbance were measured (Biotron Libra S60) at absorbance of 600 nm. Subsequent plots of growth versus OD (600 nm) were generated. A glucose assay and total protein assay were conducted to determine the usage of glucose by the *S. cerevisiae* cells and the total protein content of the cell biomass (Supplementary Information (S2) in Supplementary Materials). The glucose assay was conducted by the 3,5-Dinitrosalicylic acid (DNS) method. Briefly, to a 3 mL of 1% DNS reagent (containing 10 g of Dinitrosalicylic acid with 2 g of phenol, 0.5 g of sodium sulphite, 10 g of sodium hydroxide and finally adding water to make up for 1 L), 3 mL of sample (growth culture) was added in a lightly capped tube. The mixture was heated at 90 °C for 15 min. Then, 40% potassium sodium tartarate solution was added into the mixture and cooled to room temperature (24 °C) with an ice bath. Once cooled, the absorbance was measured at 575 nm to obtain the total glucose content of the sample. The protein assay was conducted by Bradford Protein Assay. Briefly, for the preparation of the Bradford reagent, 100 mg Coomassie Brilliant Blue G 250 was dissolved in 50 mL of 95% ethanol and added to 100 mL of 85% (*w/v*) phosphoric acid. The dye solution was filtered through a Whatman no. 1 filter paper prior use. Protein samples (100  $\mu\text{L}$ ) was added to 5 mL dye reagent and incubated for 5 min. The absorbance was measured at 595 nm.

#### 2.2.3. Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth microdilution method according to the standard procedure CLSI-M07-A9, 2008, developed by the Clinical and Laboratory Standard Institute (CLSI), USA. Two-fold, serially-diluted ILs were dispensed into each of the 96 wells of a standard microdilution plate. The direct

colony suspension method was used for inoculum preparation. *S. cerevisiae* suspension was prepared by direct transfer of colonies from 24 h agar plates to Mueller Hinton broth. Suspensions were adjusted using counting chamber to contain approximately  $1 \times 10^8$  CFU mL<sup>-1</sup>. A 100 µL of each suspension was mixed with 100 µL serially diluted tested compound in 96 microdilution plate according to the microdilution method. Two-fold dilutions of ILs were prepared and added accordingly into the wells from 100,000 ppm to 196 ppm. Un-inoculated wells were prepared as control samples. Plates were incubated at 35 °C for 24 h. The minimum (inhibitory) concentration was defined as the lowest concentration of test compound producing no visible growth.

#### 2.2.4. Screening of ILs Concentration

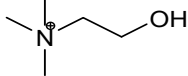
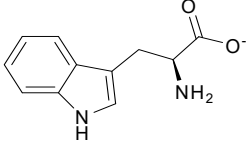
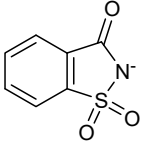
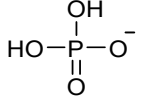
To determine the suitable concentration of each ILs to be used in the combination, an initial screening test was carried out. A stock concentration from 10,000 ppm to 196 ppm (by 2-fold dilution) were prepared for each of the ILs. A 96-well microplate was used, with each well added with all 3 ILs with a different set of IL combination. The wells were then added with 0.5 mL McFarland standardized *S. cerevisiae* culture and incubated for 18 h. The OD at 600 nm was then taken for each well and the results were tabulated. Based on the result, the best concentration for each ILs to be used in combination with each other was determined by taking the well that has the highest growth compared to the others. This concentration was then used for all the other experiments.

### 3. Results and Discussion

The ILs used in this study were choline-based with various anions. Table 1 depicts the thermophysical properties of the synthesized ILs. The NMR characterization was done to determine the purity of the synthesized ILs (Supplementary Information (S1) in Supplementary Materials).

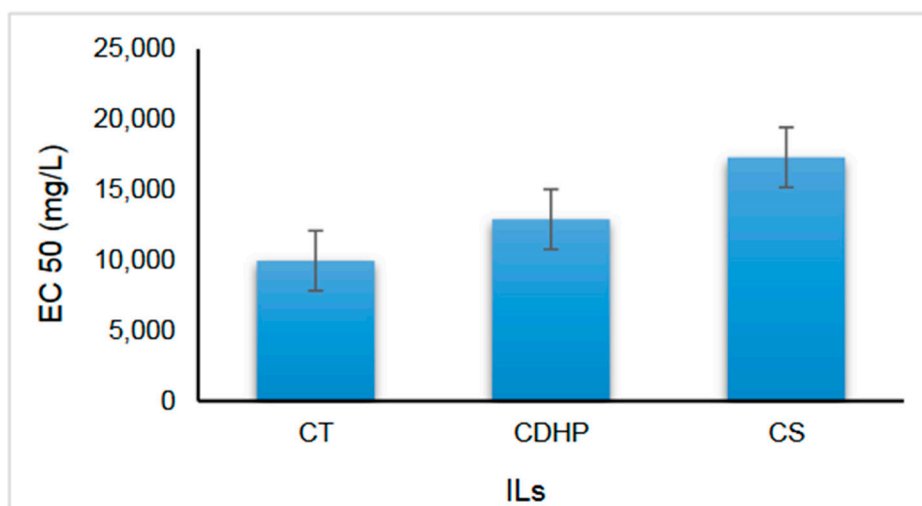
The main idea for choosing these three ILs was to replace the components of commercial yeast cultivation media. Thermogravimetric analysis was conducted to ensure that the decomposition temperature of the ILs were well above 121 °C. This is to ensure that no decomposition should be observed when the compounds were autoclaved during media preparation.

**Table 1.** Thermophysical properties of ILs used in this experiment.

ILs		Abbreviation	Molecular Weight, (g mol <sup>-1</sup> )	Physical Appearance	Thermal Properties	
Cation	Anion				TGA Decomposition Temperature, T <sub>onset</sub> (°C)	DSC Glass Transition Temperature, T <sub>g</sub> (°C)
 Choline	 Tryptophanate	CT	307.39	Brown, viscous liquid	187.55	-26.88
	 Saccharinate	CS	286.35	Light yellow, viscous liquid	248.82	-62.33
	 Phosphate	CDP	201.16	White, viscous liquid	229.85	-24.63

### 3.1. Toxicity of ILs

Toxicity studies are often reported for *E. coli*, *Lactobacillus species*, *Clostridium sporogenensis*, filamentous fungi and extremophiles but are lacking in yeast species [38,42]. Figure 1 shows the EC<sub>50</sub> values with respective 95 percent confidence levels obtained in the fit of data.



**Figure 1.** EC<sub>50</sub> (mg L<sup>-1</sup>) values of 3 ionic liquids (ILs) tested.

Measure of ILs toxicity were based on the parameters by Passino and Smith, 1987 [43] who ranked toxicity to be: 100–1000 mg L<sup>-1</sup> as being practically harmless; 10–100 mg L<sup>-1</sup> as being moderately toxic; 1–10 mg L<sup>-1</sup> being slightly toxic; and 0.1–1 mg L<sup>-1</sup> being highly toxic. For all ILs tested, dose response curves were obtained and EC<sub>50</sub> values were calculated in mg L<sup>-1</sup> (ppm). All the EC<sub>50</sub> data were in the range of “practically harmless”. Although all three ILs depicted values of “practically harmless”, the EC<sub>50</sub> values of CT (9964 mg L<sup>-1</sup>) were lower than the other two ILs. This is probably due to the high level of toxicity within the anions, which comes from tryptophan amino acid. Although tryptophan is an amino acid, it was found to have toxicity towards biological organisms—particularly towards higher organisms where they have found that continuous ingestion of L-tryptophan has been associated with multi-systemic syndrome [44]. The IL CS had the highest EC<sub>50</sub> value, indicating it being the least toxic among the three ILs tested. CS is known to have low toxicity due the precursors: Choline chloride, which has been well documented as animal feed; and saccharinate, which is known to be used as an artificial sweetener [45]. CDHP has been used to increase the thermal stability of proteins and is employed widely in the field of molecular biology [46]. CDHP has also shown to have low interaction between the alkyl chain of the lipids contained within the cell wall [46]. It was found that the toxicity toward these ILs was contributed by the interaction between the salt and the alkyl chain of the cell wall, destabilizing them and causing cell lysis. The results obtained in this study is in agreement with Vrikkis et al. (2009) [22] who reported that CS had low toxicity whereas CDHP was proven to have a stimulatory hormetic effect on cell growth.

In the past however, reports on the compatibility of ILs to *S. cerevisiae* were reported, and this included that of the study of Ouellet et al. (2011) [32] who noticed growth inhibition of *S. cerevisiae* by emim[OAc]. Authors then concluded that the cationic imidazolium moiety is the primary source of inhibition. Another study by Mehmood and team in 2015 [34], found that the anion associated to the imidazolium cation seemed to play a deleterious role whereby the *S. cerevisiae* growth was severely affected by the acetate anion. A study by Zhu et al. (2013) [31], also noted that bmim[Cl] at high concentrations (>10<sup>-3</sup> g L<sup>-1</sup>) inhibited cell growth. In a similar study by [47], EC<sub>50</sub> of 530 mg L<sup>-1</sup> was obtained with bmim[Cl] as the ILs and *Saccharomyces cerevisiae* as the organism. This was very much lower than that of this study which indicates that the ILs chosen in this study to be far more harmless to *S. cerevisiae*. Authors then concluded that bmim[Cl] had a relatively strong inhibition on yeast growth.

In a recent study by Matsuda and team (2019) [7]—using *S. pombe* yeast cells—they observed that the aforementioned yeast cells retained higher bioactivity in choline-based ILs than imidazolium-based ILs. This was supported by studies using molecular dynamics by Yoo et al. (2016) [48] that observed the penetration of long alkyl chain of an imidazolium cation into the phospholipid bilayer led to the disruption of cell membranes. Choline, on the other hand, did not disrupt the membrane structure.

Generally, choline based ILs are non-toxic in nature [7,49,50]. They are ubiquitous in the exoplasmic or outer leaflet of a cell membrane as phosphatidylcholine. To design a biocompatible IL, the precursor plays a major role. Due to this, choline based ILs are much favored within the scientific community for its relatively low toxicity towards living organisms [23,24,40,45,51].

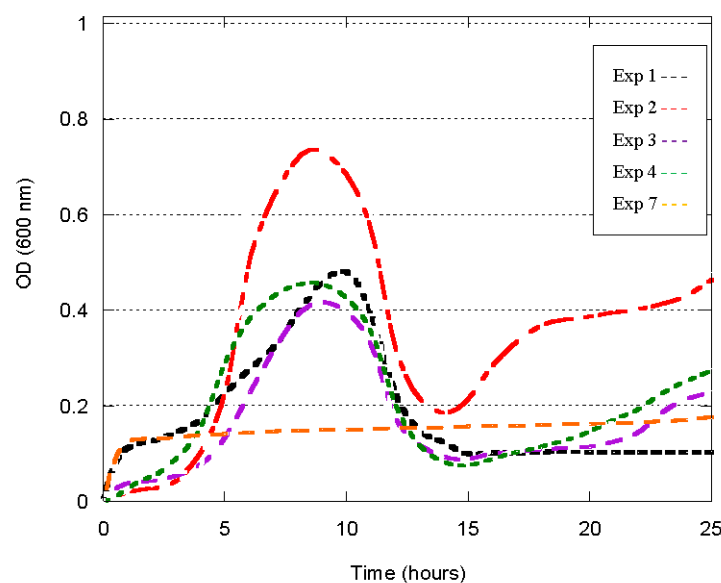
### 3.2. Growth Profile

Various sets of experiments were conducted and the ratio of ILs to YPD media is depicted in Table 2. Subsequent experiments were based on the experiment number as in Table 2.

**Table 2.** Design of Experiment for *S. cerevisiae* cultivation media composition.

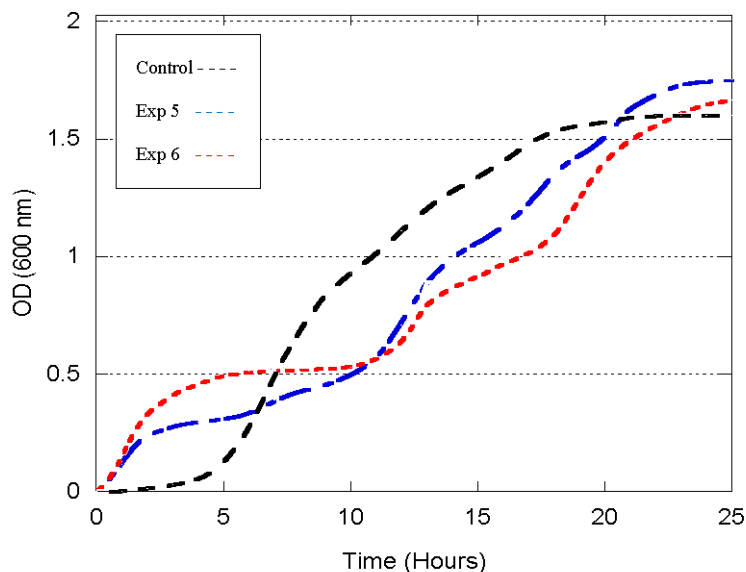
Experiment	Media Constituent	Ratio
1	CT + CDHP + CS	2:1:2
2	Yeast Extract + CDHP + CS	1:1:2
3	CT + Peptone + CS	2:2:2
4	CT + CDHP + Glucose	2:1:2
5	Yeast Extract + PEPTONE + CS	1:2:2
6	Yeast Extract + CDHP + Glucose	1:1:2
7	CT + Peptone + Glucose	2:2:2

The growth profile of *S. cerevisiae* was observed with respect to all the media compositions and Figure 2 was generated. Figure 2 depicts the growth curve of all the tested media composition (except Exp 5 and Exp 6). The control experiment depicted a typical growth pattern with a distinct lag and rapid exponential phase (Figure 3; death phase was not accounted for in Figure 2). Growth patterns of all experiments were evaluated over the same period in the same conditional settings (30 °C, 200 rpm). It is noteworthy to mention that the addition of ILs to experimental set ups did not increase the turbidity of the culture nor decrease the pH of the medium.



**Figure 2.** *S. cerevisiae* growth curve of two experimental set-up against the control (Exp 5: yeast, peptone, CS; Exp 6: yeast, CDP, glucose).





**Figure 3.** Growth of *S. cerevisiae* in various experimental set-up against control experiment.

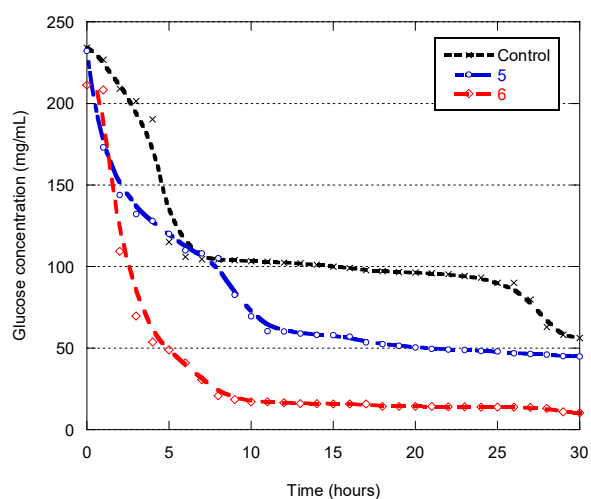
Figure 3 represents the growth pattern of Exp 5 and Exp 6. Both the curves did not represent an ideal lag phase. The growth pattern of Exp 5 and 6 displayed a prominent diauxic shift which was not very visible in the case of the control. This occurrence was also observed with Mehmood et al., (2015) [34], when employing 2 ILs; emim[OAc] and emim[CH<sub>3</sub>PO<sub>4</sub>] in *S. cerevisiae* growth media [34]. This phenomenon occurs when *S. cerevisiae* switch from fermenting the sugar source (glucose in Exp 6 and CS in Exp 5) and begin their respiratory metabolism [38]. After 20 h, we observed higher growth in media containing ILs. Exp 5 seemed to depict the highest growth values of up to 13% compared to the control. When compared to the control, the growth of the yeast in ILs containing media (primarily media with IL CDHP and CS), showed an increase in growth during the lag phase. It showed that the growth stimulation was due to the utilization of ILs as a nutrient source or the solubilization for the other media components [15]. Another prominent observation is that, media composition containing CT had low growth profiles. This is seen in Figure 3 where Exp 1, 3, 4, and 7 showed growth three times lower than the control. This tallies with the EC<sub>50</sub> values whereby although CT was said to be “practically harmless”, the values were lower than the other ILs tested. The toxicity of the ILs was evident in delaying the growth or causing a complete inhibition. These similar findings were observed with Dipeolu et al. (2009) [15], where two ILs tested, [EtOHNMe<sub>3</sub>][Me<sub>2</sub>PO<sub>4</sub>] and DMEAA increased the growth rate of *Clostridium sporogenes* by 28%. However, authors mentioned that growth inhibitions were observed at high ILs concentrations. Other studies also confirmed that yeast strains showed an increased growth in the presence of 1% [emim][OAc] [35]. In another study by Ganske et al. [52], authors mention that an increase in ILs concentration of up to 10% did not disrupt the growth of *Pichia pastoris*—a methylotrophic yeast. The ILs [bmim]Cl<sup>-</sup> at high concentrations (>10<sup>-3</sup> g L<sup>-1</sup>) was seen to inhibit the growth of *S. cerevisiae* by reducing its budding rate; however at lower concentrations (<10<sup>-3</sup> g L<sup>-1</sup>), its impact was negligible.

The hormesis data exhibited in this investigation is coherent with several studies that have been conducted using the model organism *S. cerevisiae*. Hormetic data is relatable to the incubation time as well as the cationic component of the ILs [53]. The hormetic phenomena of microorganisms are often seen particularly in cases of short chained ILs [33]. Among them include a study conducted by Vasylykova et al. [26] whereby they investigated the hormesis effect of yeast against hydrogen peroxide. They concluded—with sufficient evidence that at low level of toxic concentration, it provided the organism with the potential to survive consequent lethal stress. In this study, after the introduction of the yeast culture into the media containing ILs, there appeared to be doubling of cells until day 5. The cells clearly did not undergo a distinct log phase. This result is similar to the work conducted by

Mehmood et al. (2015) [34] where they have reported that no lag phase was observed when their media culture was introduced with [emim][MeO(H)PO<sub>2</sub>]. It has been seen that the culture containing CDHP has an accelerated growth (Exp 6). Choline is an essential nutrient and serves many physiological roles due to its incorporation in the membrane components, signaling molecules and neurotransmitters. A previous study conducted by [54] has reported an inverted U-shaped dose response curve for choline in cholinergic drug studies. Authors suggested that the biphasic effect of the dose-response curves indicates that more than one intracellular process are involved. A biphasic dose response curve indicates complex physiological action, which gives rise to the hormetic effect. Studies by Sendovski et al. (2010) [39] presented growth curve with the addition of ILs (bmim[NTf<sub>2</sub>], MPPyr [NTf<sub>2</sub>], and OMA[NTf<sub>2</sub>]). They mentioned that an addition of up to 20% ILs facilitated better growth values, and the author added that the growth rate was unaffected by the presence of ILs. However, omim[PF<sub>6</sub>] completely inhibited cell viability and it is speculated that the long hydrophobic chain penetrates the cell membrane and kills the *Saccharomyces cerevisiae* cell. In another study by Nancharaiyah et al., the authors [33] found that growth of *P. putida* was 400% higher in medium supplemented with 0.5 g L<sup>-1</sup> emim[OAc].

### 3.3. Glucose Utilization Profile

To assess the growth of the yeast further, a glucose consumption profile was conducted using the DNS glucose determination method. Figure 4 depicts the glucose concentration (mg L<sup>-1</sup>) against time.



**Figure 4.** Glucose concentration of two experimental set-up against the control (Exp 5: yeast, peptone, CS; Exp 6: yeast, CDP, glucose).

The control sample shows a steady glucose reduction consistent with *S. cerevisiae* growth curve. It shows a gradual decrease of glucose with depletion after 28 h. As for the experimental set-ups it is interesting to note that the glucose level decreased fast for the initial 5 days. This was particularly seen in the case of Exp 6, where CDHP was present. Exp 5 showed higher glucose concentrations than Exp 6 but not as high as the control. It should be noted that since *S. cerevisiae* is a type of strain that is able to grow anaerobically, it sometimes shows unusual behavior during its aerobic phase. This similar case was noticed in a few studies where ILs was incorporated in growth media [31,34,38]. In a study conducted by Dickinson et al. [38], they reported that the Y133-IIL strain had a greater growth and sugar conversion in the presence of 1% [emim]Cl. Another study by Zhu et al. [31], reported that in the presence of [bmim]Cl, the yields of fermentable sugars were lower while the growth rate was higher. In contrast, Mehmood and team [34] observed that the glucose consumption was poor with addition of ILs in the *S. cerevisiae* growth media. Our study clearly indicates that the glucose consumption was higher and more rapid when the culture media was added with ILs. The results obtained also supported the initial claim that CDHP—through a certain mechanism—increases the metabolism of

yeast and subsequently boosts the growth rate of the yeast culture. It is possible to conclude that the cells were able to utilize the ILs as a nutrient source.

#### 4. Conclusions

This is the first time choline-based IL was introduced as a synthetic media for the growth of *S. cerevisiae*. The ILs were successfully synthesized and we reported their thermophysical properties. The most significant observation is that the incorporation of the three-choline based ILs (CS, CT, and CDHP) increased the growth and enhanced the metabolism of *S. cerevisiae*. Growth of *S. cerevisiae* with the media composition of yeast extract, peptone, and CS at a ratio of 1:2:2 showed an improvement of 13%. The hormesis data exhibited in this investigation is coherent with several studies that have conducted similar experiments using the model organism *S. cerevisiae*. The hormetic effect however increased the glucose utilization profile of *S. cerevisiae* in Exp 5 (Yeast: Peptone: CS) at a ratio of 1:2:2 and Exp 6 (Yeast: CDHP: Glucose) at a ratio of 1:1:2 media composition.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2227-9717/7/7/471/s1>.

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#### Abbreviation

emim[Cl]	1-ethyl-3-methylimidazolium chloride
bmim[Cl]	1-butyl-3-methylimidazolium chloride
emim[OAc]	1-ethyl-3-methylimidazolium acetate
emim[CH <sub>3</sub> PO <sub>4</sub> ]	1-ethyl-3-methylimidazolium methylphosphonate
DMEAA	<i>N,N</i> -dimethylethanolammonium acetate
[EtOHNMe <sub>3</sub> ][Me <sub>2</sub> PO <sub>4</sub> ]	2-hydroxyethyl trimethyl ammonium dimethyl phosphate
emim[MeO(H)PO <sub>2</sub> ]	1-ethyl-3-methylimidazolium methylphosphonate
omimPF <sub>6</sub>	1-methyl-3-octylimidazolium hexafluorophosphate
YPD	Yeast Extract-Peptone-Dextrose
FDA	Food and Drug Administration

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