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ORIGINAL PAPER

Anaerobic digested sludge: a new supplementary nutrient source for ethanol production

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Abstract Effluent sludge from an anaerobic digester was used as a source of nitrogen, phosphate, sulfur, and other nutrients in the culture medium of ethanol production by the yeast *Saccharomyces cerevisiae*. Several pretreatments (mechanical, chemical, thermal, and thermo-chemical) were performed on the anaerobic digested sludge (ADS) to make the nutrients accessible to the yeast cells. Preliminary experiments revealed that *S. cerevisiae* is not able to assimilate the carbon content of the ADS. However, when glucose was added to the medium, ethanol production was observed. The yield of ethanol using untreated ADS was only 10 % of the theoretical yield, but alkaline pretreatment improved it up to 43 %. By separating the hydrolysate of alkaline-treated ADS from the suspended solids, the ethanol yield from the supernatant was further improved up to 65 % of theoretical yield. Alkaline-treated ADS exhibited competitive performance with the mixture of yeast extract and mineral salts in ethanol fermentation.

Keywords Bioethanol · Anaerobic digested sludge · Sludge management · Pretreatment · *Saccharomyces cerevisiae*

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Abbreviations

AC	Acidic pretreatment
ADS	Anaerobic digested sludge
AL	Alkaline pretreatment
ALTH	Combined alkaline and thermal pretreatment
CFU	Colony forming units
PE	Pretreatment efficiency
SCOD	Soluble chemical oxygen demand
TCOD	Total chemical oxygen demand
TDS	Total dissolved solids
TH	Thermal pretreatment
TS	Total solids
UL	Ultrasonic pretreatment
UNS	Untreated sludge
WAS	Waste activated sludge
WWS	Wastewater sludge
WWTP	Wastewater treatment plant

Introduction

Rapid urbanization, especially in the developing countries, and the need for appropriate water and sanitation, necessitated large investments on constructing more wastewater treatment plants ($WWTP_S$) in the last few decades. Activated sludge process is the most widely used process for the treatment of industrial and municipal wastewaters (Motlagh and Goel 2014). In a typical activated sludge process, roughly one-third of the organic carbon from wastewater is converted to waste activated sludge (WAS) (Tchobanoglous et al. 2003). This has caused one of the greatest environmental concerns which come from the accumulation of WAS (Brar et al. 2009) and directed research efforts toward development of new alternative options for either minimization or proper handling of sludge.

Since WAS contains various nutrients such as carbon, nitrogen, and phosphorous (Tyagi and Lo 2013), utilization of sludge as a replacement for some of the nutrients in the culture media of biological production of value-added products has been investigated as an alternative method (Brar et al. 2009). Thus, biotechnological products such as biopesticides (Yezza et al. 2005), biochemicals such as lactic acid (Ma et al. 2014), and biofuels such as biohydrogen (Ting and Lee 2007) have been produced using WAS as a nutrient source for the involved microorganisms. Cheung and Anderson (1997) tried to produce ethanol from the cellulose content of primary sludge of WWTPs, via simultaneous saccharification and fermentation (SSF). Additionally, using chemical and physical processes, researchers have tried to produce products such as bioplastics (Chua et al. 2003), biofertilizers (Ben Rebah et al. 2007), and biodiesel (Siddiquee and Rohani 2011) from WAS.

Despite all of these researches, anaerobic digestion has remained the dominant large-scale process which is widely applied to transform organic carbon in WAS into methane and reduce the amount of biosolids to be disposed of (Bolzonella et al. 2012, Wang et al. 2014). However, this approach solves the problem only partially. After the anaerobic digestion process, sludge volume and mass are greatly reduced but a new sort of waste, called digestate, is produced which needs further treatment and disposal. It contains biodegradable and recalcitrant organic compounds, pathogens, heavy metals, and other inorganic constituents. However, this sludge can still be considered a source of nutrients and energy, which could be recovered using economically viable approaches. In fact, the digestate has a higher proportion of mineral nitrogen and less decomposable organic matter (Tambone et al. 2010). Nowadays, common treatment technologies to manage this waste are landfilling, combustion, and land application (Zhang et al. 2014).

In many countries, very strict limitations have been set to minimize the disposal of sludge to the landfills due to increasing greenhouse gas emissions (CH_4 and CO_2) and leaching of heavy metals to water and soil from landfills (Kelessidis and Stasinakis 2012). Combustion of sludge is also usually a difficult process due to the high ash and moisture content of ADS (Roy et al. 2011). The potential agricultural use of ADS is also under question because it may result not only in the transfer of resistant bacteria into the environment but also in the propagation of antibiotic resistance genes and have an impact on the bacterial communities of the receiving ecosystem (Calero-Caceres et al. 2014). However, as a matter of fact, after anaerobic digestion of WAS, the produced ADS is still a valuable source of organic carbon, nitrogen, phosphorous, sulfur, as well as some inorganic compounds such as silicates and aluminates, which can be reutilized for value-added production purposes (Zhang et al. 2014).

The hypothesis of the present work was investigating the possibility of utilizing the ADS as an alternate nutrient source for producing bioethanol. Bioethanol is currently the most abundant renewable in the global fuel market which is industrially produced mainly from corn and sugar cane (Soccol et al. 2010). In order to reduce the cost of ethanol production and overcome the "food versus energy" conflict, lots of researchers have focused on finding new carbon source for bioethanol production including agricultural residues and municipal or industrial wastes (Jafari et al. 2011; Jeihanipour and Taherzadeh 2009; Sheikh et al. 2013; Jeihanipour and Bashiri 2015).

For optimum growth of yeast and bioethanol production, yeast extract plus some other synthetic chemicals is often used as a nutrient source in laboratory-scale fermentation (Jeihanipour and Taherzadeh 2009). However, the high cost of this synthetic medium limited its application in industrial process. Thus, new nutrient alternative suitable for bioethanol fermentation is explored by researchers (Asachi et al. 2011). To the best of our knowledge, while this article is being written, there is no scientific report on using ADS as a nutrient source in fermentation processes to produce bioethanol. The ADS is a difficult substrate to be consumed by microorganisms since most of its organic matters have been converted to more complex compounds in the digester (Manara and Zabaniotou 2012). Thus, strong pretreatments are required to break down its structure and release the remained organic matters.

The goal of this study was to investigate the possibility of producing bioethanol by *Saccharomyces cerevisiae* from the excess sludge of anaerobic digester, produced in a municipal WWTP. However, it was expected and confirmed by preliminary experiments that the carbon content of ADS cannot be consumed by *S. cerevisiae*. Therefore, the pretreated ADS, enriched by an additional carbon source, i.e., glucose, was used as culture media for production of ethanol via an anaerobic fermentation process. The effect of different treatments and inhibition of sludge on ethanol production were also investigated.

Materials and methods

Microorganism and inoculum preparation

The yeast strain *S. cerevisiae,* CEN.PK113-7D used in this study was grown on agar slants containing (g/l): D-glucose, 20; yeast extract, 10; soy peptone, 20; and agar, 20, at 30 °C for 36 h and then maintained at 4 °C until being used. The inoculum was prepared using fermentation media containing (g/l): D-glucose, 40; NH_4Cl, 7.5; K_2HPO_4, 3.5; $MgSO_4{\cdot}7H_2O$, 0.75; $CaCl_2{\cdot}2H_2O$, 1; yeast extract, 5. A volume of 25 ml of medium was inoculated by a loopful of the yeast grown on an agar plate and then incubated aerobically at 30 °C and 180 rpm for 20 h.

Sludge analyses and preparation

The ADS, used in this study, was obtained from north WWTP of Isfahan, Iran. Upon receiving, it was stored in several 100-ml polypropylene containers, at −18 °C, and prior to use, the wet ADS was thawed and sterilized at 121 °C for 30 min.

Sludge pretreatment

In order to release nutrients exist in the ADS suspended solids, various pretreatments including acid hydrolysis (AC), alkaline hydrolysis (AL), thermal hydrolysis (TH), ultrasonication (UL), and the combination of alkaline and thermal hydrolysis (ALTH) were applied and their efficiency was compared.

Acid (AC) or alkaline (AL) pretreatments were conducted on 25 ml of sterilized ADS in glass bottles at a pH of 2 or 11 by the aid of a few drops of 1 N H_2SO_4 or NaOH solution, respectively, followed by incubation at 37 °C for 2 h.

Thermal pretreatment (TH) was also carried out on the sterilized ADS, in an autoclave for 1 h at 121 °C (Barjenbruch and Kopplow 2003).

The ultrasonic pretreatment (UL) was performed using a 300-W sonication apparatus (Hielscher UP200S, Teltow, Germany) with a probe diameter of 14 mm. The sonication was carried out at room temperature for 1 h (Pham et al. 2009) while the probe was immersed 2 cm into 25 ml of sterilized ADS in a 50-ml falcon tube.

The combination of alkaline and thermal pretreatment (ALTH) was performed on 25 ml of ADS in a 50-ml bottle the same as mentioned above. The alkaline treatment was performed prior to the thermal treatment.

The pH of all pretreated samples was adjusted at 7 ± 0.5 by the aid of a few drops of 1 N H_2SO_4 or NaOH solution, and then they were sterilized for 20 min at 121 °C to be prepared for inoculation.

The pretreatment efficiency (PE) was calculated using Eq. (1) (Foladori et al. 2010):

$$\mathrm{PE} = \frac{(\mathrm{SCOD} - \mathrm{SCOD}_0)}{(\mathrm{TCOD} - \mathrm{SCOD}_0)} \times 100 \quad (1)$$

SCOD soluble chemical oxygen demand of pretreated sludge (mg/l); $SCOD_0$ soluble chemical oxygen demand of untreated sludge (mg/l); *TCOD* total chemical oxygen demand of untreated sludge (mg/l).

Aerobic fermentation

In order to determine the biomass production ability of *S. cerevisiae* using nutrients of the ADS, aerobic cultivation was performed. Pretreated or untreated ADS was mixed with deionized water (final solid concentration of 10 g/l) and glucose solution (final glucose concentration of 40 g/l). The samples were then inoculated by *S. cerevisiae* inoculum (4 % v/v), making an initial cell concentration of about 6×10^5 cell/ml, followed by incubation at 30 °C and 180 rpm for 20 h. The cell concentration of *S. cerevisiae* before and after incubation was measured using the colony-forming units (CFU) method. The growth ability was calculated based on the initial (X_0) and final (X) viable yeast cells in the culture (Eq. 2).

$$\text{Growth ability} = X/X_0 \quad (2)$$

Anaerobic fermentation

Anaerobic fermentation was carried out in batch glass bottles (working volume of 25 ml) with butyl rubber seals and aluminum caps. A volume of 5.2 ml of pretreated or untreated sludge was mixed with deionized water (final sludge solid concentration of 10 g/l) and glucose solution (final glucose concentration of 40 g/l), and then the bottles were sealed and sparged with pure nitrogen gas for 10 min to provide anaerobic condition (Jeihanipour et al. 2010). The bottles were sterilized at 121 °C for 20 min, inoculated (4 % v/v resulting in an initial yeast cells concentration of about 10^6 cells/ml), and then incubated at 30 °C and 180 rpm for 2 days. Samples were withdrawn every 12 h for glucose and ethanol analyzes. While sampling, the extra pressure due to produced CO_2 inside bottles was released. The percentage of theoretical yield of ethanol was calculated using Eq. (3):

$$\text{Percentage of theoretical yield of ethanol} = \frac{\frac{g\ \text{Produced ethanol}}{g\ \text{Initial glucose}}}{\text{Theoretical yield of ethanol}} \times 100 \quad (3)$$

The theoretical yield of ethanol from glucose is 0.51 g ethanol/g glucose.

It should be noted that, since it is well known that *S. cerevisiae* is only able to consume six-carbon sugars, glucose (40 g/l) was added to ADS as a carbon source in all the experiments. However, the capability of the microorganism for converting the carbon content of the pretreated and untreated ADS into ethanol under anaerobic condition was examined.

Moreover, in order to investigate the effect of total solids of ADS on ethanol production and evaluate its inhibitory effect toward *S. cerevisiae*, three sets of fermentations were performed. In the first series, treated ADS with a final solid concentration of 10 g/l was added to the fermentation media containing (g/l): D-glucose, 40; NH_4Cl, 7.5; K_2HPO_4, 3.5; $MgSO_4{\cdot}7H_2O$, 0.75; $CaCl_2{\cdot}2H_2O$, 1; yeast extract, 5. The result was compared to the media without sludge, called control sample. In the second series, different concentration of treated ADS (0, 5, 10, and 30 g/l solid concentration) was added to glucose solution (final glucose concentration of 40 g/l) and then inoculated to investigate the effect of total solid concentration of sludge on fermentation. In the third one, the liquid phase and solid phase of treated ADS were separated by centrifuging at 4000 rpm for 20 min and used as a supplementary nutrient in ethanol fermentation.

Analysis methods

In order to identify the amount of total carbon, hydrogen, nitrogen, and sulfur of the ADS, it was dried at 70 °C and then ground by a household mill (Moulinex^{TM}). The dried ADS was analyzed by a CHNS analyzer (LECO 923, LECO, MI, USA). Moreover, total solids, volatile solids, fixed solids, total dissolved solids, phosphorous (PO_4^{3-}), ammonium nitrogen (NH_4^+–N), and chemical oxygen demand (COD) were determined according to the APHA standard methods (Rice et al. 2012). The heavy metals analysis was performed using a Flame Atomic Absorption Spectrometer (Varian 220 FS, Varian, Palo Alto, CA).

The liquid samples taken from the fermentation media were analyzed after centrifugation at 10,000 rpm for 10 min. The ethanol concentration was measured by a gas chromatograph (Agilent 6890 N, Agilent Technologies Inc., CA, USA) equipped with an FID detector. Analytes were separated on an HP-INNOWAX column (60 m × 0.32 mm i.d., 0.5 μm film thickness). The carrier gas was nitrogen with a flow rate of 3.3 mm/min. The detector and injector temperatures were set at 200 °C and 160 °C, respectively. The initial oven temperature was adjusted at 60 °C. The oven temperature was increased to 80 °C at a ramp of 10 °C/min and then to 150 °C at a rate of 60 °C/min. The oven temperature was held at 150 °C for 1 min.

All experiments were performed at least in duplicate, and the results are presented as averages with deviations of less than 7.0 %.

Results and discussion

Characterization of the ADS

The physicochemical properties of the ADS, used in this study, are shown in Table 1. The pH of the ADS was around 8.0, and its total solids (TS) and total dissolved solids (TDS) were 48.5 and 5.0 g/l, respectively. Therefore, the suspended solids could be estimated as 43.5 g/l. Volatile solids and fixed solids concentrations were measured as 24.3 and 25.0 g/l, respectively. Besides, CHNS analysis showed that the ADS consists of 31 % carbon, 5 % hydrogen, 4 % nitrogen, and 1 % sulfur. Total ammonia–nitrogen and phosphorous as PO_4^{3-} were also measured as 573.3 and 657.1 mg/l, respectively (Table 1). Additionally, the most abundant heavy metals in the ADS were Fe (10,900 mg/l), Zn (550 mg/l), Cu (290 mg/l), and Mn (200 mg/l), whereas the concentrations of Pb (73 mg/l), Ni (34 mg/l), Cr (35 mg/l), and Cd (1 mg/l) were not high, compared to the sludge analyzed in other studies (Table 2). Total chemical oxygen demand (TCOD) was also measured as 18,262 mg/l of which 7066 mg/l was in soluble form (Table 1).

Pretreatments efficiency in releasing the nutrients of the ADS

In order to release the organic matters of ADS, several pretreatments (mechanical, chemical, thermal, and thermochemical) were performed. The pretreatment efficiency (PE), a criterion of efficiency of the treatment to increase the soluble COD, was calculated using Eq. (1). The results are shown in Table 3. The amount of NH_4^+–N and PO_4^{3-} measured after pretreatments are also presented in this table. Most of the enhancement was obtained after ALTH and UL treatment with pretreatment efficiency of 66.9 % and 37.4 %, respectively. The TH, AC, and AL treatments could also solubilize 21.7, 16.7, and 16.4 % of COD, respectively. Besides, ALTH could release more

Table 1 Physicochemical characteristics of ADS

Physical characteristics	ADS	Chemical characteristics	ADS
Total solids (g/l)	48.50 ± 0.71	Total carbon (%)	30.85 ± 0.08
Total dissolved solid (g/l)	5.00 ± 0.05	Total nitrogen (%)	3.47 ± 0.01
Total suspended solids (g/l)	43.50 ± 0.71	Total hydrogen (%)	4.73 ± 0.00
Volatile solids (g/l)	24.34 ± 0.94	Total sulfur (%)	0.76 ± 0.04
Fixed solids (g/l)	25.00 ± 0.94	Ammonia nitrogen (mg/l)	573.34 ± 0.05
pH	7.93 ± 0.08	Phosphorous as PO_4^{3-} (mg/l)	657.14 ± 0.05
		COD (mg/l)	18.260 ± 10
		SCOD (mg/l)	7.070 ± 8

Table 2 Comparison of heavy metals content of ADS of Isfahan wastewater treatment plant with the sludge of other countries

Heavy metal	ADS used in this study (mg/l)	ADS in Denmark (mg/l) (Xiang et al. 2000)	ADS in Hong Kong (mg/l) (Marchioretto et al. 2002)
Fe	10,900	18,800–24,500	72,200
Zn	550	1320–2320	2823
Cu	290	745–1050	255
Mn	200	280–420	–
Pb	73	185–205	57
Cr	34	345–495	663
Ni	35	20–40	622
Cd	1	2–3	–

Table 3 Efficiency of different pretreatments (PE) on ADS and the amount of NH_4^+–N and PO_4^{3-} released after each pretreatment and the growth ability of *S. cerevisiae* on pretreated and untreated ADS

Pretreatment	PE[a] (%)	NH_4^+–N (mg/l)	PO_4^{3-} (mg/l)	Growth ability[b]
ALTH	66.9	513.34 ± 0.05	517.86 ± 0.05	Na[c]
AL	16.4	420.00 ± 0.05	507.14 ± 0.05	19.9
AL[d]	16.4	420.00 ± 0.05	507.14 ± 0.05	0.35
TH	21.7	Na	Na	17.2
AC	16.7	Na	Na	12.1
UL	37.4	Na	Na	0.4
UNS	–	373.34 ± 0.05	457.14 ± 0.05	8.5

[a] Calculated using Eq. (1)

[b] Calculated using Eq. (2)

[c] Not analyzed

[d] Cultivation without glucose

ammonia–nitrogen and phosphorous, i.e., 513.3 and 517.9 mg/l, respectively, in comparison with AL treatment which released 420.0 mg/l NH_4^+–N and 507.1 mg/l PO_4^{3-}. This method of analysis and formula of calculation of PE are usually used to evaluate the effect of a treatment on excess activated sludge followed by anaerobic digestion to reduce the sludge volume (Foladori et al. 2010). Therefore, measuring the released COD can somehow predict whether the pretreatment has been effective to increase the yield of methane; however, this COD cannot necessarily be consumed by an ethanol producer microorganism like *S. cerevisiae*.

Aerobic growth of *S. cerevisiae* on ADS and the effect of pretreatment

In order to investigate the growth ability of *S. cerevisiae* on the ADS, a cultivation medium consisted of glucose as carbon source and pretreated or untreated ADS (UNS) as the source of supplementary nutrients was used and the

growth ability was calculated using Eq. (2). Whereas the growth ability of *S. cerevisiae* on the UNS was only 8.5, AL, TH, and AC treatments significantly increased the growth ability of *S. cerevisiae* to 19.9, 17.2, and 12.1, respectively. However, ultrasonication of ADS led to a significantly lower growth ability of *S. cerevisiae*, i.e., 0.4, compared to the UNS (Table 3). These results confirmed that with the help of a suitable pretreatment, ADS could be considered as a promising substitute nutrient for yeast extract and other synthetic nutrients in cultivation media of *S. cerevisiae*.

Effect of pretreatment of ADS on the ethanol yield of *S. cerevisiae*

Since the easy-to-digest nutrients exist in the sludge used in the present study have already been consumed during biological processes taking place inside the digester, performing an appropriate pretreatment on the sludge is a key factor in releasing the nutrients and making them accessible for the microorganisms.

After 48 h of fermentation, the ethanol yield in the control was obtained as 84 % of the theoretical yield (Fig. 1). At the same condition, the AL and ALTH treatment led to the highest ethanol yield, i.e., 43 and 33 % of the theoretical yield, respectively, while the AC, TH, and UL treatment and UNS resulted in a lower ethanol yield of 16, 3, 2, and 10 % of the theoretical yield, respectively (Fig. 1).

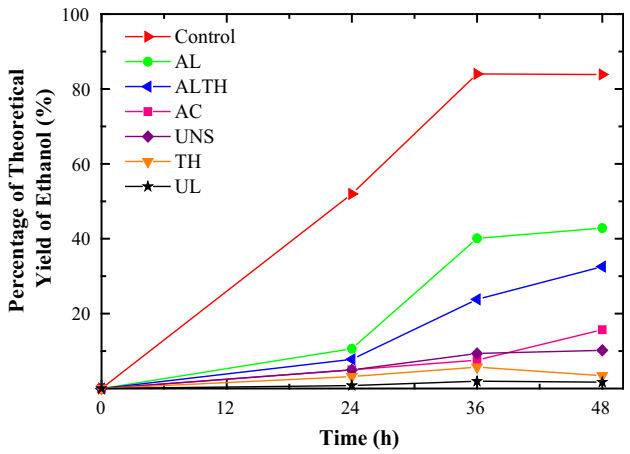

Fig. 1 Ethanol yield (as a percentage of the theoretical yield) for the treated ADS (total solid concentrations of 10 g/l) and control sample during 48 h of fermentation

On the other hand, although the amount of soluble oxygen demand after ALTH and also UL was significantly higher than that achieved by AC, TH, and AL treatments (Table 3), the ethanol yield was the highest for the AL treatment and the least for the one treated by UL (Fig. 1). These conflicting results can be explained by considering the pretreatment mechanisms. There are several studies indicating the possibility of heavy metal solubilization after acid hydrolysis of sludge (Babel and del Mundo Dacera 2006), which might adversely affect the growth of microorganisms (Wang and Chen 2006) and ethanol yield.

The ion Fe^{2+} has been shown having mutagenic effect on the *S. cerevisiae*. Indeed, excess free iron has the potential to disrupt cellular processes through several mechanisms such as participating in oxidation–reduction chemistry inside the cell, leading to generation of hydroxyl radicals and other reactive oxygen species (Philpott et al. 1998). Besides, Mn^{2+} can cause a significant reduction in the biomass yield of *S. cerevisiae* (Blackwell et al. 1998). Therefore, with respect to the heavy metal content of the utilized sludge in the present study (Table 2), it can be concluded that acidic pretreatment could solubilize these heavy metal ions and subsequently, negatively affect yeast growth and ethanol production.

In the case of thermal treatment, which results in cell degradation, the intracellular content is released and some chemical and physical reactions occur between them, causing the formation of inhibitory intermediate compounds and recalcitrant (Wilson and Novak 2009). At high temperatures, carbohydrates and released amino acids get involved into maillard reactions leading to the formation of melanoidine, which are extremely recalcitrant compounds (Carrère et al. 2010). The thermal treatment done in this study might have resulted in toxic compounds formation and the reduction of ethanol yield consequently. However, when thermal process is combined by alkaline treatment, these negative effects are reduced considerably, making the ALTH a second candidate for the highest ethanol yield in comparison with the stand-alone AL. This result is in accordance with other findings, suggesting AL and ALTH being the best ones for solubilizing the nutrients of sludge (Yan et al. 2013).

The contradictory results in COD solubilization and ethanol yield for the UL might also be due to the potential of the ultrasound waves in releasing the Mg^{2+} and Ca^{2+} ions existing among the extracellular polymeric substances, the main component of ADS (Pilli et al. 2011). Indeed, the microorganism growth rate might have been negatively affected by these ions.

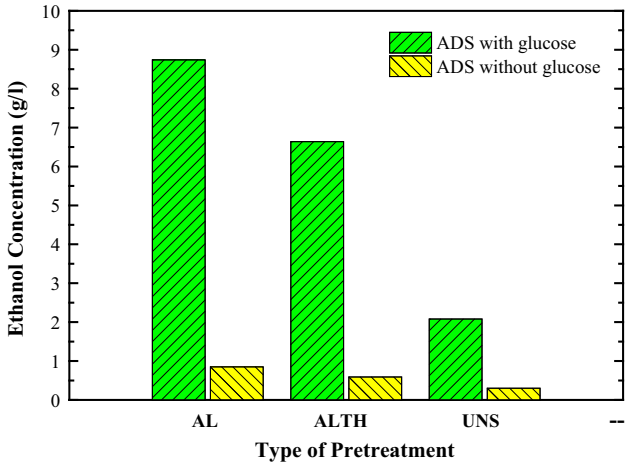

Fig. 2 Ethanol concentration for the pretreated and untreated ADS (total solid concentrations of 10 g/l) with and without glucose after 48 h of fermentation

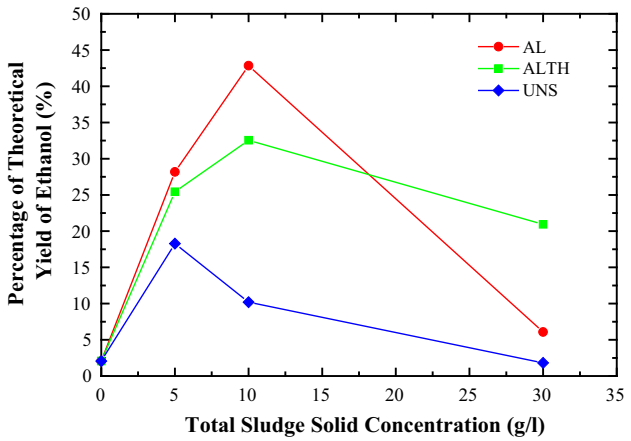

Fig. 3 Ethanol yield (as a percentage of the theoretical yield) for pretreated and untreated ADS containing different total solid concentrations after 48 h of fermentation

Importance of the presence of both glucose and ADS in the media of fermentation

In the absence of glucose, after 48 h of fermentation, the concentration of ethanol in the mediums containing 10 g/l total solid of AL- and ALTH-treated ADS was obtained as 0.8 and 0.6 g/l, respectively (Fig. 2), while by adding 40 g/l glucose to the medium the ethanol concentration at the same conditions was increased to 8.7 and 6.6 g/l, respectively (Fig. 2). In the case of UNS, the concentration of ethanol after 48 h fermentation was 0.3 and 2.1 g/l, with no glucose and with 40 g/l glucose, respectively. These results showed that neither the pretreated ADSs nor the untreated one led to a considerable accumulation of ethanol in the absence of glucose. Therefore, for the rest of the experiments, glucose as a carbon source was added to the culture medium and the ADS served as a source of nutrients other than carbon. This result was in accordance with other works of literature (Ma et al. 2014; Kobayashi et al. 2005).

In addition to this, in order to convey the significance of ADS on ethanol yield, *S. cerevisiae* was inoculated to a sludge-free medium, consisted of only glucose and water under anaerobic condition. It can be inferred from Fig. 3 that when total solid of sludge was 0, the ethanol concentration was negligible (nearly 2 % of theoretical yield). In fact, this minimal yield is obtained as a result of nutrients within the inoculum itself. In other words, ethanol production depends highly on the presence of other nutrients as well as glucose, which is merely supplied by ADS in this study.

Effect of total solids of ADS on ethanol production

Three sets of experiments were performed to study the inhibitory effect of total solid content of ADS on the production of ethanol. In the first series, a standard synthetic media, i.e., the mineral salts solution used in inoculum preparation with a glucose concentration of 40 g/l, was supplemented with 10 g/l total solid of AL- and ALTH-treated ADS to examine the effect of sludge on the efficiency of ethanol production by yeast. The results showed that the ALTH-treated ADS has no inhibitory effect on ethanol production and the yield, i.e., 86 % of theoretical yield, is similar to the ethanol yield of the control medium containing no sludge (Table 4). However, the addition of 10 g/l of AL-treated sludge reduced the ethanol yield up to 64 % of theoretical yield (Table 4). Therefore, it can be concluded that ALTH treatment reduced the inhibitory effect of ADS but produced fewer nutrients, consumable by *S. cerevisiae*, compared to the AL treatment.

The second set was designed to compare ethanol yields at different total solid concentrations (i.e., 0, 5, 10, and 30 g/l) of ADS. In the cultivation medium, besides sludge,

Table 4 Comparison of ethanol yield (as a percentage of theoretical yield) for the solid phase and liquid phase of ADS as the only supplementary nutrient sources and also ADS supplemented with mineral salts

Pretreatment	Yield of ethanol after 48 h fermentation (% of theoretical yield)		
	Liquid phase of ADS	Solid phase of ADS	ADS + Mineral salts + Glucose
ALTH	62.54	10.15	85.54
AL	65.49	8.28	63.53
Control	–	–	83.87

there was 40 g/l glucose. Data presented in Fig. 3 reveal that for untreated ADS, total solid concentration higher than 5 g/l causes inhibitory effect, while for AL- and ALTH-treated sludge the inhibitory effect was observed at total solid concentrations of above 10 g/l. Therefore, there is an optimum level for the total solids of sludge for ethanol production, which may change depending on the employed treatment. This was in accordance with other literature (Vidyarthi et al. 2002).

In the third series of the experiments, the solid phase of ALTH- and AL-treated ADS was separated from the liquid phase and both were separately tested as nutrient sources for ethanol production (Table 4). When only solid part of the ALTH- and AL-treated ADS was used, the ethanol yield of 10 and 8 % of the theoretical yield was respectively obtained (Table 4). However, supplementing the media with the supernatant of those sludges resulted in, respectively, 63 and 65 % of the theoretical yield of ethanol after 48 h of fermentation. The solid particles may exhibit inhibitory effects on the growth and ethanol production by aggregating and slowing down the transport of nutrients to the cell (de Lourdes Tirado Montiel et al. 2001). Thus, the whole process can be improved by using the supernatant of pretreated sludge. This is because the released nutrients are more likely to transfer to the microorganism due to the unanimous medium provided by the supernatant.

Economic perspective

There is a lack of data for economic evaluation of ADS-based ethanol in the litrature since no one has so far used ADS as a supplementary nutrient source for ethanol production. However, in some articles, second-generation ethanol from lignocellulosic residues is investigated from this point of view (Macrelli et al. 2012). For instance, by targeting the ethanol production of 45 l per dry ton of sugarcane and estimating the costs as follows: enzymes (0.341 US $/l), acid (0.08 US $/l), base (0.02 US $/l), water consumption (0.045 US $/l), vinasse sales (−0.003 US $/l), labor, maintenance, and insurance (0.148 US $/l), electricity (0.357 US $/l), and capital cost (0.56 US $/l), the minimum ethanol selling price would be calculated as 1.548 US $/l (Macrelli et al. 2012). Although this estimation contains the cost of pretreatments in which either of chemicals or energy is required, the price of other nutrients such as yeast extract and synthetic salts which can increase the costs substantially is not included. Therefore, it is obvious that by replacing ADS as a nutrient supplementary source, the final selling price of ethanol would decline considerably. Moreover, by integrating the ADS-based ethanol plant with WWTPs, it is possible to reduce the disposal costs of ADS as well as its environmental risks.

Conclusion

In the present study, the digested sludge (ADS), an unavoidable by-product of the WWTPs, was used as a supplementary nutrient to replace yeast extract and other nutrients, i.e., mineral salts, in fermentation media of ethanol production. Besides, this could be a more sustainable option in sludge management via using ADS nutrients in biological production of value-added products (Brar et al. 2009). The obtained results showed that the order of pretreatment efficiency to increase the ethanol yield was AL > ALTH > AC > TH > UNS > UL. It is worth reminding that apart from the sludge in the media, there were only glucose and water. In other words, in one liter of culture media, 5 g yeast extract plus 12.75 grams other nutrient chemicals was replaced by 206 ml ADS containing 10 grams total solid, and finally, around 10 g ethanol (0.25 g/g glucose) was produced after AL treatment of ADS. This result is very promising and could be improved further by optimizing the pretreatment parameters and fermentation conditions. Moreover, there is an optimum point for sludge with different total solid concentrations depending on the pretreatment type. For instance, while the concentration of 10 g/l was obtained as an optimum point for AL and ALTH, the optimum point of 5 g/l was gained for UNS. Besides, the results showed that the supernatant of ALTH- and AL-treated ADS has a better potential for ethanol production in comparison with its solid phase and can improve the ethanol yield up to 63 and 65 % of the theoretical yield, respectively.

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